The KIMONO-study

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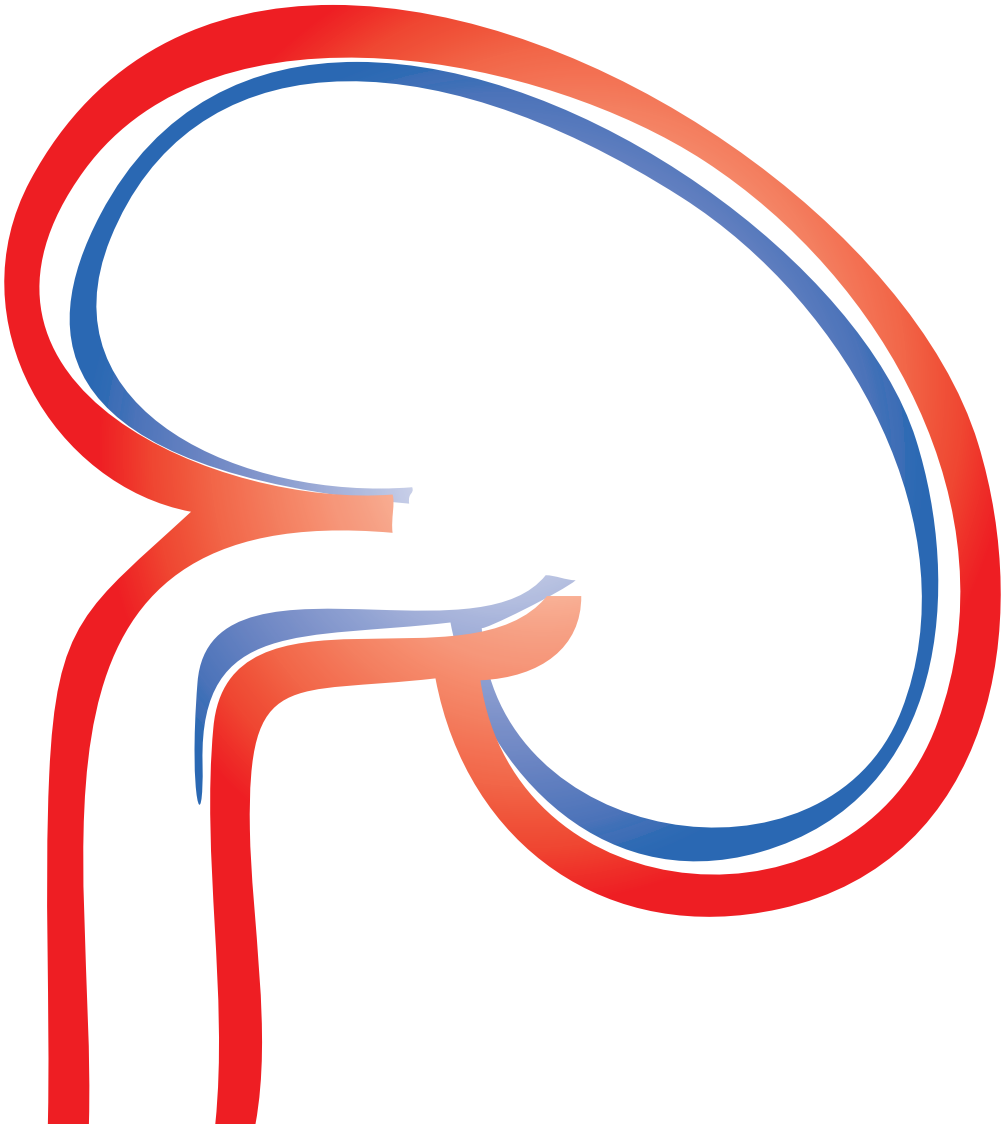
Rik Westland

On the development of renal injury in children with a solitary functioning kidney



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Science moves with the spirit of an adventure characterized both by youthful arrogance and the believe that the truth, once found, would be simple as well as pretty

James D. Watson

Aan mijn ouders

VRIJE UNIVERSITEIT

The KIMONO-study

On the development of renal injury in children with a solitary functioning kidney

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	dr. M.F. Schreuder

Contents

Chapter 1

Clinical implications of the solitary functioning kidney

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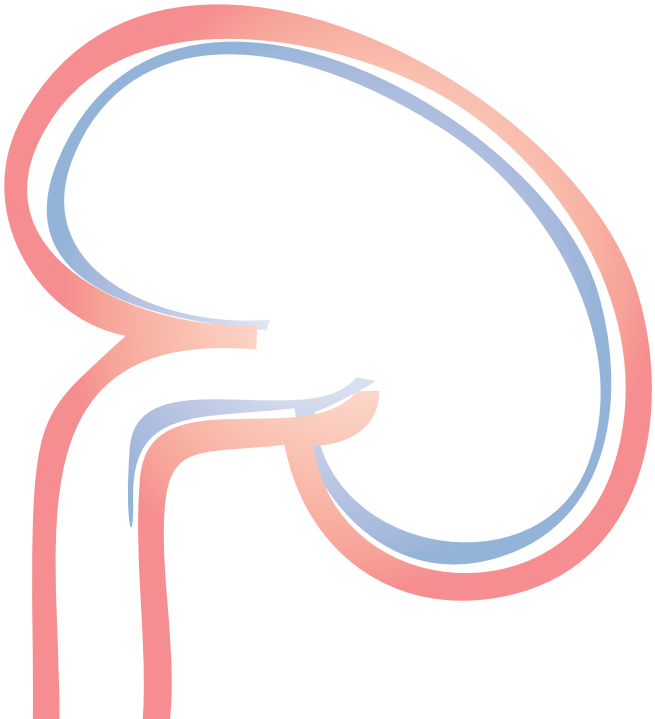


ABSTRACT

Congenital anomalies of the kidney and urinary tract are the major cause of end-stage renal disease in childhood. Children with a solitary functioning kidney form an important sub-group of congenital anomalies of the kidney and urinary tract patients, and a significant fraction of these children is at risk for progression to chronic kidney disease. However, challenges remain in distinguishing patients with a high risk for disease progression from those patients without a risk for disease progression. Although it is hypothesized that glomerular hyperfiltration in the lowered number of nephrons underlies the impaired renal prognosis in the solitary functioning kidney, the high proportion of ipsilateral congenital anomalies of the kidney and urinary tract in these patients may further influence clinical outcome. Pathogenic genetic and environmental factors in renal development have increasingly been identified, and may play a crucial role establishing a correct diagnosis and prognosis for these patients. With fetal ultrasound now enabling prenatal identification of individuals with a solitary functioning kidney, an early evaluation of risk factors for renal injury would allow for differentiation between patients with and without an increased risk for chronic kidney disease. This review describes the underlying causes and consequences of the solitary functioning kidney from childhood together with its clinical implications. Finally, guidelines for follow-up of solitary functioning kidney patients are recommended.

INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) are the predominant cause of end-stage renal disease in childhood.[1] One important condition in the spectrum of CAKUT is the solitary functioning kidney, which can be congenital or acquired following unilateral nephrectomy in childhood. Although both types of solitary functioning kidney are associated with chronic kidney disease (CKD) and end-stage renal disease,[2] early differentiation between patients with and without an increased risk for CKD is challenging.[3] Because of the implementation of routine fetal ultrasound screening in most developed countries, patients with a solitary functioning kidney are increasingly identified before birth. This identification not only implies that clinicians will be more often confronted with questions regarding the prognosis of this specific condition, but also that these children can be clinically monitored from birth onward. In this review, we consider causes and consequences of the solitary functioning kidney from childhood. We will specifically focus on the diagnostic and prognostic implications for patients with a solitary functioning kidney.

CAUSES

Renal development

Definitive human renal development is initiated at the fifth gestational week and characterized by complex interactions between the outgrowing ureteric bud (UB) of the mesonephric duct (from which the renal pelvis, ureter, and lower urinary tract originate) and the metanephric mesenchyme (MM; from which the renal parenchyma originates).[4] As a result, nephrons are formed until the 34th to 36th gestational week without the possibility of additional nephron formation later in life.[5] This finding implies that the total of number of nephrons at birth, approximately 900,000 nephrons per kidney with a high interindividual variability,[6] should last the entire life span of an individual.

Failure of interaction between the MM and the UB perturbs normal renal development, resulting in different forms of CAKUT.[7] Bilateral absence of functioning renal tissue is considered lethal based on the associated pulmonary hypoplasia (Potter sequence). Unilateral renal agenesis (URA), which defines unilateral nonformation of the kidney, has an estimated worldwide incidence of 1 in ~2,000 births.[8] Because differentiation from renal aplasia cannot be made in daily clinical practice, URA is generally used as a term for either clinical entity, although a study has suggested that renal aplasia may be the leading cause of a congenital solitary kidney (1 in ~1,300 births[9]). URA should be differentiated from abnormal or incomplete renal development, which leads to renal hypodysplasia or a non-functioning kidney, which can be seen in multicystic dysplastic

kidney (MCDK; worldwide incidence 1 in ~4,300 births[10]). However, it must be noted that the diagnosis URA could derive from the spontaneous (prenatal) involution of MCDK or renal hypodysplasia.[8] It is estimated that about 5% of MCDKs show complete involution before birth.[10]

In the case of URA or MCDK, the solitary functioning kidney is congenital. However, a solitary functioning kidney can also be acquired after nephrectomy because of various renal diseases such as CAKUT (e.g. pelviureteric junction obstruction (PUJO), vesicoureteric reflux (VUR), megaureter and duplex kidney).[11] Renal malignancies, trauma or renovascular disease may also underlie a solitary functioning kidney, but these causes are outside the scope of this review.

Ipsilateral CAKUT

CAKUT are phenotypically variable and may affect several segments of the urinary tract simultaneously.[12] The high proportion of ipsilateral CAKUT (i.e. renal malformations on the side of the solitary functioning kidney) in solitary functioning kidney patients is a clear illustration of this phenomenon. Based on two systematic reviews and meta-analyses of the literature describing over 2,000 patients with URA and MCDK,[8,10] nearly one in three patients has ipsilateral CAKUT, including VUR, PUJO, a megaureter or a duplex kidney. Although the prevalence of ipsilateral CAKUT in patients with an acquired solitary functioning kidney has not yet been established, a large cohort study suggests even higher proportions.[13]

Genetic factors

Normal kidney and urinary tract development requires a temporally and spatially coordinated interaction between the UB and the MM.[14] Different molecules expressed in either or both of these compartments have been identified through gene targeting in mice.[15] As a result, any insult (genetic or environmental) that disrupts this reciprocal induction, can lead to different forms of CAKUT. In fact, this strict cross-talk between the UB and the MM provides the rationale for the pleiotropic effect that genetic mutations or environmental factors can have on the determination of different forms of CAKUT. There is a bulk of evidence that implicates genetic factors in the pathogenesis of the CAKUT-phenotypes underlying a solitary functioning kidney. Familial aggregation has been reported in about 10% of cases (more frequently with an autosomal dominant mode of inheritance with incomplete penetrance, but recessive and X-linked families have been reported as well[12]). With the exception of rare syndromic forms of CAKUT, the mutations underlying these familial forms are largely unknown.[12] The three genes most commonly implicated in non-syndromic forms of CAKUT are *PAX2* (encoding for a nuclear transcription factor involved in early nephrogenesis), *HNF1B* (encoding for a transcription factor originally implicated in the renal cysts and diabetes syndrome [Online Mende-

lian Inheritance in Man 137920]), and *DSTYK* (encoding for a dual-specificity serine/threonine and tyrosine kinase; recently identified as a positive regulator of FGF signaling during kidney development).[16] However, many other genes have been implicated in CAKUT-phenotypes underlying a solitary functioning kidney (Table 1.1). Heterozygous mutations in all these genes, nevertheless, account for, at most, 10-20% of patients with kidney malformations.[17,18]

TABLE 1.1. Genes most commonly implicated in CAKUT-phenotypes leading to a solitary functioning kidney.

Isolated forms of renal hypodysplasia/CAKUT (including solitary functioning kidney)				
OMIM	Gene symbol	Phenotype	Syndrome	Mode of inheritance
*612666	*DSTYK*	RHD, PUJO	-	dominant
#137920	*HNF1B*	RHD	Renal cysts and diabetes syndrome (RCAD)	dominant
#120330 / #191830	*PAX2*	RHD	Papillorenal syndrome	dominant
#219000	*FRAS1, FREM1*	Renal agenesis, RHD	Fraser syndrome	dominant / recessive
*611559	*UPK3A*	Renal adysplasia/urogenital adysplasia	-	dominant
*112262	*BMP4*	RHD, renal agenesis	-	dominant
*191830	*RET*	Renal agenesis	Hirschsprung's disease	dominant
Syndromic forms of renal hypodysplasia/CAKUT (including solitary functioning kidney)				
#113650	*EYA1, SIX1, SIX2, SIX5*	Renal agenesis, RHD	Branchio-oto-renal syndrome	dominant
#107480	*SALL1*	Renal agenesis, RHD, VUR, renal ectopia	Townes-Brocks (Branchio-oto-renal-like syndrome)	dominant
#607323	*SALL4*	Renal ectopia, CAKUT	Okihiro syndrome	dominant
#308700	*KALL1, FGFR1*	Renal agenesis, RHD	Kallman's syndrome	dominant
*610132	*VANGL1*	Renal agenesis, RHD, renal ectopia	VACTERL / Caudal regression syndrome	dominant
*142994	*MNX1*	Renal agenesis, RHD, renal ectopia, VUR	VACTERL/ Caudal regression syndrome / Currarino syndrome	dominant
#118450	*JAG1, NOTCH2*	RHD, MCDK	Alagille syndrome	dominant
#214800	*CHD7*	Renal agenesis, RHD, renal ectopia, VUR	CHARGE syndrome	dominant
#146255	*GATA3*	RHD	Hypothyroidism, sensorial deafness	dominant
#161200	*LMX1B*	Renal agenesis	Nail-patella syndrome	dominant
#122470	*NIPBL*	Renal agenesis, RHD	Cornelia de Lange syndrome	dominant
#180849	*CREBBP*	Renal agenesis, RHD, VUR	Rubinstein-Taybi syndrome	dominant
#147920	*MLL2*	VUR, RHD, renal ectopia	Kabuki-syndrome (VUR)	dominant

TABLE 1.1 (continued)

OMIM	Gene symbol	Phenotype	Syndrome	Mode of inheritance
#146510	*GLI3*	Renal agenesis, RHD	Pallister-Hall syndrome	dominant
#130650	*KIP2*	RHD, VUR	Beckwith-Wiedemann syndrome	dominant
#181450	*TBX3*	Renal agenesis, RHD	Ulnar-Mammary's syndrome	dominant
#270400	*DHCR7*	RHD, cysts, renal agenesis, VUR	Smith-Lemli-Opitz syndrome	recessive
#214100	*PEX-family*	RHD, cysts	Zellweger syndrome	recessive
#277000	*WNT4*	Renal agenesis, RHD, renal ectopia	Rokitansky syndrome	dominant
#300209	*GPC3*	RHD, cysts, VUR	Simpson-Golabi-Behmel syndrome	X-linked

CAKUT, congenital anomalies of the kidney and urinary tract; CHARGE, coloboma of the eye, heart defects, atresia of the nasal choanae, retardation of growth/or development of genital and/or urinary abnormalities, and ear abnormalities and deafness; MCDK, multicystic dysplastic kidney; RHD, renal hypodysplasia; PUJO, pelviureteric junction obstruction and VUR, vesicoureteric reflux.

CAKUT are among the most common birth defects in humans (1 in ~600 births),[19] and present in over 20% of newborns with chromosomal abnormalities,[20] indicating that kidney development is particularly sensitive to gene dosage. Consistently, in a recent study on 522 children with renal hypodysplasia (including solitary functioning kidney) we identified 72 different copy-number disorders in 87 patients (~17%), implicating rare, submicroscopic deletions and duplications that disrupt coding elements as a major cause of renal hypodysplasia and confirming the extreme genetic heterogeneity of this disease.[21]

Environmental factors

Environmental factors that disturb renal development include medications administered during pregnancy (e.g. angiotensin-converting-enzyme [ACE] inhibition, dexamethasone, antiepileptic drugs and aminoglycosides),[22] intra-uterine growth restriction and maternal diseases such as diabetes.[23] Moreover, drug administration in the prematurely born neonate with a solitary functioning kidney can have detrimental effects on nephrogenesis and glomerular filtration rate (GFR), especially when administered before the 28th gestational week.[5] The most commonly used drugs that disturb nephrogenesis are aminoglycosides and non-steroidal anti-inflammatory drugs.[22] Finally, observational studies report a boy predominance in solitary functioning kidney patients, as well as a left-side predominance.[24] The reasons for these differences are not fully understood yet. Future studies are needed to clarify the exact mechanisms of renal (mal)development and the factors leading to a solitary functioning kidney.

CONSEQUENCES

Hyperfiltration hypothesis

The clinical importance of a reduced nephron number has been described in the hyperfiltration hypothesis by Brenner and coworkers[25,26] more than three decades ago. Based on experiments in rats with five sixths renal mass reduction, these landmark studies showed that a reduced functional nephron number results in compensatory glomerular hypertension and enlargement of remnant nephrons indicating glomerular hyperfiltration.[25,26] Glomerular hyperfiltration may lead to glomerulosclerosis and sets a vicious cycle of additional reduction in nephron number (Figure 1.1). As a consequence, the nephrectomized animals showed high incidences of hypertension and proteinuria in the early stages and an ongoing decline in GFR in the long run.[25]

Individuals with a solitary functioning kidney from childhood have, by definition, a reduction in renal mass for a prolonged period of time and may, therefore, be at risk for hyperfiltration injury (Figure 1.1). Animal studies confirmed that a compensatory increase in nephron number is present in congenital solitary functioning kidney.[27,28] Nevertheless, the total nephron number identified is still lower than in two kidney controls

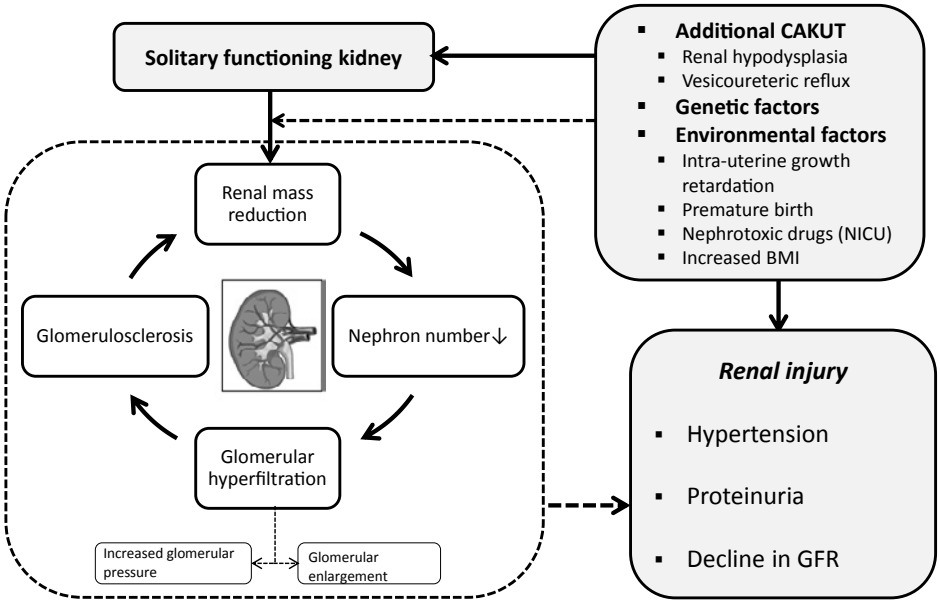


FIGURE 1.1. Possible mechanisms leading to renal injury in patients with a solitary functioning kidney. Glomerular hyperfiltration caused by insufficient nephron number could be one explanation for this impaired outcome. Nephron number could also be affected by associated congenital anomalies of the kidney and urinary tract as well as genetic and environmental factors. BMI, body mass index; CAKUT, congenital anomalies of the kidney and urinary tract; GFR, glomerular filtration rate and NICU, neonatal intensive care unit.

TABLE 1.2. Nephron number in different species with a solitary functioning kidney.

Publication	Species	Model	Age at study	Nephron number in % of two kidney controls	Glomerular size in % of two kidney controls
Larsson et al.[47]	Rat	Nephrectomy (during postnatal nephrogenesis)	55-65 days (adulthood)	52%	NR
Bhathena et al.[59]	Human	URA	34.5 years (adulthood)	NR	175%
Abellan et al.[60]	Rabbit	Nephrectomy *(in utero)*	30 days of gestation	NR	115%*
Mesrobian[61]	Mouse	URA	8 weeks (adulthood)	NR	113%
Douglas-Denton et al.[27]	Sheep	Nephrectomy *(in utero)*	27-34 days (infancy)	72%	74%
Van Vuuren et al.[28]	Pig	URA	26 weeks (adulthood)	75%	152%

*, Measured by total glomerular surface area. NR, not reported and URA, unilateral renal agenesis.

(Table 1.2). Compensatory renal hypertrophy of the solitary kidney can be identified *in utero* in 90% of individuals.[29] This hypertrophy indicates renal adaptation to kidney mass reduction and may, therefore, be beneficial at birth. However, a prolonged increase in nephron and kidney size may lead to stretch-induced glomerular cell activation, fibrosis and vasoconstriction as well as tubular cell nephrotoxity.[30]

Keller et al.[31] provided indirect evidence of the glomerular hyperfiltration hypothesis in patients with primary hypertension; the patients had fewer nephrons and an increased glomerular volume compared with patients without hypertension.[31] Recently, another study showed that urinary albumin is weakly associated with markers of hyperfiltration in solitary functioning kidney patients,[32] indicating that factors other than glomerular hyperfiltration likely contribute to the development of CKD.

Because the reduction in renal mass in solitary functioning kidney patients is smaller compared with the Brenner animal model, *in vivo* methods to determine nephron number are necessary to establish the direct association between glomerular numbers and the level of glomerular hyperfiltration in affected individuals. Some of these methods are in a promising developmental phase,[33] and, when available, they will allow for the direct testing of the hyperfiltration hypothesis in humans.

Clinical outcome

Human studies

The long-term outcome of individuals with a solitary functioning kidney from childhood has been a topic of extensive debate,[3] fueled by the conflicting results of observational studies.[2,11,34-44] All of these studies vary with respect to type of solitary functioning kidney

studied, inclusion criteria (e.g. age at follow-up, age of diagnosis and normal renal ultrasound) and methods to measure blood pressure, proteinuria and GFR. Furthermore, observational studies are susceptible to selection and ascertainment bias. The number of longitudinal prospective studies on the clinical outcome of solitary functioning kidney patients is limited because of the decades required for follow-up.[45] A longitudinal study on renal outcome in CAKUT showed that 20-50% of solitary functioning kidney patients were on renal replacement therapy at the age of 30 years.[2] Compared with a reference group, the risk for an impaired renal outcome was even higher when VUR was present (hazard ratio: 7.50, 95% confidence interval 2.72 to 20.68). Results from another retrospective study on URA patients showed that 47% of individuals developed hypertension, 19% of individuals had proteinuria, 13% of individuals had an impaired GFR, and 4% of individuals died of renal failure.[37] Similar findings were identified in adults with an acquired solitary functioning kidney.[36] Gonzalez et al.[39] showed that obesity is associated with an increase in serum creatinine and the development of proteinuria in adults with a solitary functioning kidney. However, all of the above mentioned studies describe a selected cohort of patients, which makes generalization of these findings to all patients with a solitary functioning kidney inappropriate. Unfortunately, individuals with a solitary functioning kidney and preserved renal function have traditionally not been followed into adulthood, and, therefore, the availability of long-term data on the clinical outcome in these patients is limited. In this regard, there is a cardinal need for long-term studies of individuals with a solitary functioning kidney with a prenatal diagnosis, because they represent the most unbiased group of patients. When available, these data will strongly assist nephrologists in differentiating patients at risk for CKD from those patients who are not at risk for CKD.

To prospectively study the prognosis of a solitary functioning kidney in childhood, we designed the KIdney of MONofunctional Origin (KIMONO)-study.[13] This longitudinal follow-up study from The Netherlands includes over 400 children with both types of solitary functioning kidney. Study subjects were routinely screened for markers of renal injury, which was defined as hypertension, (micro)albuminuria and/or decline of GFR. The use of antihypertensive and antiproteinuric medication during follow-up was monitored. Recent analyses showed that nearly one in three patients with a solitary functioning kidney has signs of renal injury at a mean age of 10 years.[11] Furthermore, we showed that GFR in these patients slowly declines from as early as 9 years of age, whereas (micro)albuminuria generally develops around 16 years of age. The median age to develop renal injury in children with either type of solitary functioning kidney was ~15 years.[13] Patients with ipsilateral CAKUT (encompassing 34% of patients) showed higher proportions of renal injury and progressed earlier to renal injury than patients without CAKUT (12.8 versus 15.9 years, respectively; $P < 0.01$).[13] Finally, 6% of children already had CKD stage 3 or higher during follow-up (congenital 4% versus acquired 9%; $P = 0.05$). Other studies that have included solitary functioning kidney patients with a

normal renal ultrasound at diagnosis (suggesting absence of ipsilateral CAKUT) report a relatively mild renal outcome.[34,40,42,43] Although the exact proportion of individuals with CKD needs to be determined, the increasing number of studies illustrates that a solitary functioning kidney should not be assumed to be harmless.

Differences between solitary functioning kidney types

Important differences in renal outcome may exist between congenital and acquired solitary functioning kidneys; the congenital type still has the potential to form new nephrons (Table 1.2), whereas with the acquired type nephrogenesis has ceased at the time of the nephrectomy. This finding may imply a higher susceptibility for pronounced glomerular hyperfiltration in acquired solitary functioning kidney patients. Abou-Jaoudé et al.[35] reported a lower GFR in children with an acquired solitary functioning kidney than in children with a congenital solitary functioning kidney (mean GFR: 95 versus 107 ml/min/1.73m^2, respectively). Nevertheless, differences between types are generally small and may also be explained by the older age in children with an acquired solitary functioning kidney.[13] It must be noted that many of the underlying causes (e.g. CAKUT) leading to an acquired solitary functioning kidney in childhood are already present before birth, whereas some patients with a congenital solitary functioning kidney have hypodysplasia of the remnant kidney. Renal compensation in nephron number, therefore, seems to be strongly overlapping between both types of solitary functioning kidneys. Large longitudinal follow-up studies are needed to differentiate the clinical outcome between solitary functioning kidney types.

Differences with uninephric kidney donors

In daily clinical practice, the outcome of patients with a solitary functioning kidney is often derived from the excellent prognosis described in adult uninephric kidney donors.[46] However, there are important differences between uninephric kidney donors and patients with a solitary functioning kidney that make such a comparison inadequate.

Larsson et al.[47] showed that the level of glomerular hyperfiltration in rats is doubled when renal mass reduction is performed during active nephrogenesis compared with later in life. Similar findings have been identified in a study with rabbits.[48] Furthermore, healthy uninephric kidney donors undergo stringent screening to ensure that a healthy kidney will remain, whereas children with a solitary functioning kidney frequently show additional anomalies and may have hypodysplasia of the solitary functioning kidney.[8,10] This finding is further underlined by the finding that not all children with a solitary functioning kidney have renal compensatory hypertrophy.[11] On the basis of the abovementioned differences between uninephric kidney donors and patients with a solitary functioning kidney, it is not justified to aggregate conclusions on outcome in both groups.

CLINICAL IMPLICATIONS

Subsequent to the ongoing debate on the clinical outcome of patients with a solitary functioning kidney from childhood, guidelines for the follow-up of these patients have not been established. However, the need for regular clinical follow-up in these patients is increasingly recognized.[11,45,49]

Based on findings of the KIMONO-study,[13] a differentiation between patients with and without a high risk for CKD should be made at diagnosis. This evaluation should focus on identified risk factors such as ipsilateral CAKUT, small renal size, low birth weight, prematurity and history of urinary tract infection. In addition, the Chronic Kidney Disease in Children (CKiD) cohort study has identified clinical (anemia, hyperphospatemia, and short stature[50]) and socioeconomic (lower income and lower maternal education[51]) risk factors that are associated with the development of CKD in children, including solitary functioning kidney patients. Risk assessment also requires imaging techniques such as renal ultrasound in all patients and, on indication, renal scintigraphy and/or a micturating cystourethrogram (for additional CAKUT including VUR) together with repeated follow-up of renal length. If imaging studies identify associated CAKUT, such as high-grade VUR or PUJO, an endoscopic or surgical approach may be indicated. Finally, identification of genetic factors to establish a molecular diagnosis and screening for CAKUT in relatives may provide additional information on the clinical outcome.

Corbani et al.[45] suggest regular clinical follow-up for hypertension, (micro)albuminuria and GFR every 3-5 years in a patient with a solitary functioning kidney without ipsilateral CAKUT. For patients with ipsilateral CAKUT, clinical follow-up should be conducted annually, and surgical correction of CAKUT must be performed when indicated.

On the basis of all available data, we present recommendations for the clinical monitoring of children with a solitary functioning kidney (Table 1.3). Because we have a limited number of factors at hand in clinical practice, it is not yet feasible to provide an individualized risk assessment and follow-up scheme. However, we feel that the available data do not allow for exclusion of any group of children with a solitary functioning kidney for clinical monitoring. We have therefore suggested a follow-up based on two factors (i.e. the presence of ipsilateral CAKUT and the presence of [signs of] renal injury). Based on these factors, follow-up for all is proposed, with a differentiation in the frequency of follow-up. No unequivocal evidence exists for any follow-up frequency, which will therefore always result in an arbitrary proposal. However, because of analysis of the Kaplan-Meier curves on renal injury in children with a solitary functioning kidney show that each year ±4% of children additionally develop signs of renal injury,[13] we feel that a yearly follow-up is justified. The proposed time intervals allow timely intervention, which mainly encompasses medications such as ACE inhibitors or angiotensin II receptor blockers (ARBs). ACE inhibitors have been shown to slow down disease progression

TABLE 1.3. Opinion-based recommendation for clinical follow-up intervals of children with a solitary functioning kidney.

	No renal injury		GFR <60 ml/min/1.73m^2 or medication for proteinuria/ hypertension
	CAKUT -	CAKUT +	
Blood pressure	One time per year	Two times per year	Two to four times per year
(Micro)albuminuria	One time per year	Two times per year	Two to four times per year
Serum creatinine / GFR	Every 5 years	Every 5 years	Two to four times per year
Ultrasound	Every 5 years*	As indicated	As indicated

Guideline for the clinical follow-up of children with a solitary functioning kidney. The presented follow-up intervals are based on risk assessment at diagnosis; 24-hour ambulatory blood pressure measurement is preferred in children and adults. Microalbuminuria should be determined in a first fresh morning sample (urinary albumin cut-off value >30mg/24h). GFR can be estimated using the commonly used Schwartz-formula. *, Last ultrasound to be performed at 15-16 years of age. CAKUT, congenital anomalies of the kidney and urinary tract and GFR, glomerular filtration rate.

in children with renal hypodysplasia.[52] Patients with a solitary functioning kidney who were diagnosed prenatally and do not show risk factors for CKD at diagnosis can be monitored less stringently. Clinicians should be specifically aware of the administration of medication that may cause acute kidney injury, such as nonsteroidal anti-inflammatory drugs and aminoglycosides. Limiting the use of drugs that disturb nephrogenesis is of particular importance in premature infants with a solitary functioning kidney.

The impact of early dietary management in infants with a solitary functioning kidney remains unclear. Rapid postnatal catch-up growth is associated with obesity in adulthood,[53] which, in turn, may deteriorate renal function in the solitary functioning kidney patient.[39] Additionally, studies by Brenner and coworkers[25,26] showed that protein restriction has a protective effect on glomerular hyperfiltration in rats. Interestingly, a combination of the Brenner hypothesis and the developmental origin of health and disease hypothesis has been proposed to cause the impaired renal outcome of very low birth weight infants.[54] One can therefore speculate that rapid catch-up growth in low-birth weight infants with a solitary functioning kidney could negatively impact renal outcome and should be discouraged. Nevertheless, this relationship between postnatal feedings strategies and renal outcome needs additional investigation.

An impaired GFR is a relatively late phenomenon in children with a solitary functioning kidney, because the first signs of renal injury generally include hypertension and (micro)albuminuria.[5] Monitoring for renal injury in children with a solitary functioning kidney and a normal initial estimated GFR therefore, should focus on blood pressure and determination of (micro)albuminuria, which can both be done noninvasively. Ambulatory blood pressure measurement to monitor for hypertension is the preferred method in children and in adults,[55] whereas microalbuminuria should be measured in a first morning void.[56] GFR in children can be estimated by the commonly used Schwartz equation, which has been shown to be precise in children with a solitary functioning

kidney.[57] The follow-up of children with a solitary functioning kidney, but without signs of renal injury, can be performed by the general pediatrician, whereas patients with renal injury and early CKD may require clinical visits by a pediatric nephrologist. Clinical visits by a pediatric nephrologist also apply to children with associated CAKUT. In the follow-up of children with a solitary functioning kidney and complex CAKUT, a multidisciplinary approach involving general pediatricians, pediatric nephrologists and pediatric urologists is mandatory in order to optimize renal outcome, particularly when surgical intervention is necessary.

Puberty seems to be a critical time in renal injury development,[13] when growth spurt and associated increases in metabolic demands may drive glomerular hyperfiltration to maintain normal GFR. Other explanations for the increase in renal injury may be found in sex hormones that could affect renal outcome.[58] After this critical time period, there is a strong need for appropriate transition of care from a pediatric to an adult nephrology setting. Ineffective transition to adult care and loss to follow-up may increase the risk for CKD in adults with a solitary functioning kidney.[49] Because CKD can be relatively symptom-free until a late stage, we emphasize the need for clinical follow-up programs of adult solitary functioning kidney patients. During these regular clinical visits, it seems mandatory to also evaluate body mass index, because obesity is an additional risk factor for the development of CKD.[39]

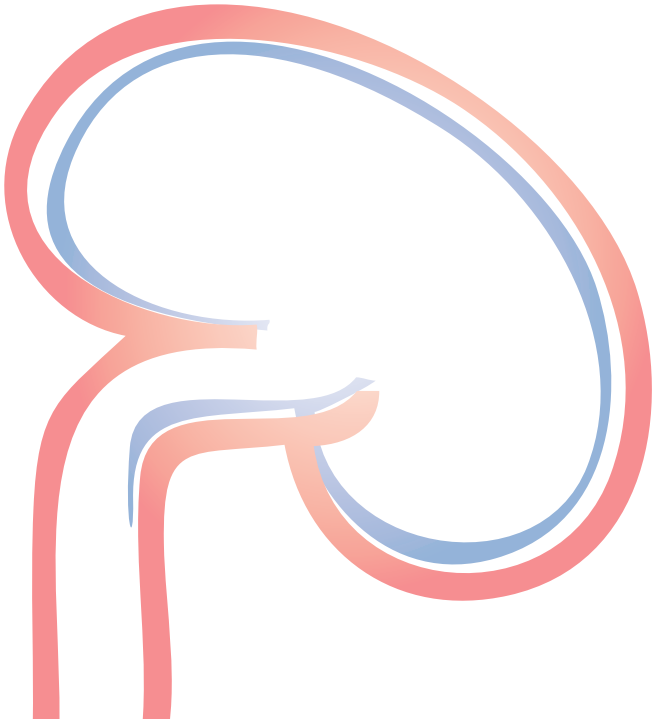
Finally, we recognize that our recommendations are limited because of the lack of consensus for long-term follow-up and the fact that longitudinal data on the clinical outcomes of individuals with a solitary functioning kidney are absent. However, we feel that these recommendations can support pediatric and adult nephrologists in the monitoring of this specific patient group.

CONCLUSION

A solitary functioning kidney from childhood implies a substantial risk for hypertension, proteinuria and progression to CKD, a statement that is supported by a well defined hypothesis, animal studies, and retrospective and longitudinal human studies. Possible explanations for this increased risk for renal injury are glomerular hyperfiltration and impaired renal development caused by genetic and/or environmental factors. Although not yet widely established, regular clinical monitoring of these patients is necessary and requires a multidisciplinary approach. In these clinical visits, it is important to differentiate between patients with and without a high risk for CKD. Future genetic studies and the development of new techniques to determine nephron number will further contribute to making this differentiation, optimizing the clinical approach for all solitary functioning kidney patients.

Chapter 2

Aims, design and outline of the thesis



Conflicting conclusions remain whether or not children with a solitary functioning kidney are at increased risk of developing chronic kidney disease (CKD) during childhood and later in life (**Chapter 1**). Animal studies have shown that renal mass reduction beyond a certain threshold leads to glomerular hyperfiltration in the remnant nephrons. In the long run, this glomerular hyperfiltration causes hypertension, microalbuminuria and a decline in glomerular filtration rate (GFR). As children with a solitary functioning kidney by definition have renal mass reduction and postnatal nephron formation is absent, they might be susceptible to the ongoing detrimental effects of glomerular hyperfiltration.

We designed the KIMONO-study (KIdney of MONofunctional Origin) to objectify the risk for renal dysfunction in children with a solitary functioning kidney. Furthermore, we investigated potential risk factors for an impaired clinical outcome in solitary functioning kidney patients. Ultimately, our main objective is to design guidelines for the clinical monitoring of children with a solitary functioning kidney.

To substantiate the risk for renal injury in solitary functioning kidney patients, we formulated the following research questions:

1. What is the incidence of a solitary functioning kidney in childhood?
2. What is the incidence of renal injury in children with a solitary functioning kidney?
3. What are the risk factors for renal injury in children with a solitary functioning kidney?
4. Are estimating equations for GFR and office blood pressure measurement accurate applications in the clinical monitoring of children with a solitary functioning kidney?
5. Which known and novel genetic factors can be identified in children with a solitary functioning kidney?

Design and outline of the thesis

In the remainder of this chapter, we present an overview of the design and methods used in the conducted studies.

By performing systematic reviews of the available literature, we investigated the worldwide incidence of the two conditions underlying a congenital solitary functioning kidney (**Chapters 3 and 4**). Unilateral renal agenesis (URA) is defined as the one-sided congenital absence of renal tissue resulting from failure of embryonic kidney formation. A multicystic dysplastic kidney (MCDK) is due to abnormal and incomplete renal development, leading to the formation of cysts and dysplastic renal tissue. MCDK frequently shows complete involution before birth, which hampers the distinction from URA. Both systematic reviews include a meta-analysis on the incidence of gender, affected side, additional congenital anomalies of the kidney and urinary tract (CAKUT) and extra-renal malformations.

Retrospective studies on the presence of renal injury were performed for all available data of children with a solitary functioning kidney (**Chapters 5, 6, 7 and 8**). To the best of our knowledge, these studies are conducted in the largest cohort of solitary functioning kidney patients known to date. As glomerular hyperfiltration in rats leads to hypertension and microalbuminuria in the short term and a decline of GFR in the long run, we formulated the definition of renal injury as the presence of hypertension, (micro)albuminuria and/or a GFR <60 ml/min/1.73m^2. Because medication such as angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) may confound the incidence of renal injury, we included the use of this "renoprotective" medication in our definition. To determine the timing of renal injury development in these children, we performed generalized estimated equation analyses (**Chapter 5**) and Kaplan-Meier survival analyses for renal injury markers (**Chapter 7**). Because the type of solitary functioning kidney, i.e. congenital versus acquired, and the presence of additional CAKUT may influence clinical outcome, we stratified for these factors in both analyses. Furthermore, we determined risk factors for the development of renal injury by using logistic regression models (**Chapter 7**). Potential risk factors studied were gender, age, type of solitary functioning kidney, additional CAKUT, side, prenatal diagnosis, body mass index (BMI), birth weight, urinary tract infections and renal length. The letters published in **Chapters 6 and 8** highlight the crucial distinction between children with a solitary functioning kidney and healthy adult uninephrectomy donors and the differences in clinical outcome between boys and girls with a solitary functioning kidney, respectively.

The cross-sectional studies described in **Chapters 9 and 10** were designed to optimize the clinical management of children with a solitary functioning kidney. The gold standard measurement of GFR in children, such as an inulin- or iohexol clearance test, is cumbersome and costly, and therefore not routinely performed in pediatric practice. As a consequence, equations to estimate GFR (eGFR) that use endogenous serum markers such as creatinine and cystatin C have been widely adopted by pediatricians. However, these estimating equations have never been tested in children with a solitary functioning kidney. In **Chapter 9**, we validated six commonly used eGFR equations in children with a solitary functioning kidney by comparing them to a gold standard GFR measurement performed by the inulin single-injection method. Furthermore, we determined the diagnostic accuracy of these eGFR equations by Bland-Altman analyses.

According to the hyperfiltration hypothesis, hypertension is an early indicator for renal dysfunction in solitary functioning kidney patients. Adequate measurement of blood pressure in the child with a solitary functioning kidney is therefore of cardinal importance. Although 24-hour ambulatory blood pressure monitoring is increasingly recognized as an invaluable tool in the diagnosis and management of hypertension in adults and children, studies in pediatric solitary functioning kidney patients are limited

in number of studied subjects and heterogeneous in design. In **Chapter 10**, we compared office blood pressure measurement to 24-hour ambulatory blood pressure monitoring in children with a solitary functioning kidney and determined the diagnostic accuracy of office blood pressure measurements.

Chapters 11 and 12 discuss the pivotal role of genetic factors in renal maldevelopment, and in the development of a solitary functioning kidney in particular. Patients with CAKUT (including solitary functioning kidney) frequently exhibit extra-renal disorders such as cardiac and gastrointestinal anomalies as well as neurocognitive defects. An early molecular diagnosis hypothetically allows an accurate prediction of the overall clinical outcome of solitary functioning kidney patients and the development of new therapeutic strategies. In **Chapter 11**, we underline the importance of studies using adequate genetic methods in the gene discovery for CAKUT by using a recently published manuscript as a template. The genetic architecture of CAKUT is extremely complex, and CAKUT has consequently been defined as "the disease of hundreds of genes". Standardization of genetic studies therefore is crucial for the identification of pathogenic genes implicated in solitary functioning kidney development. We propose a standardized approach in **Chapter 12**, in which the presence of rare point mutations in the three genes most commonly implicated in CAKUT, i.e. *HNF1B*, *PAX2* and *DSTYK*, as well as structural copy-number variants (CNVs) were determined in 80 children from the KIMONO cohort. Furthermore, we performed a systematic *in silico* analysis and immunofluorescence studies in the embryonic mouse kidney to define genes implicated in the development of a solitary functioning kidney.

We conclude this thesis by proposing guidelines for the clinical management of children with a solitary functioning kidney, and discuss directions for future studies in **Chapter 13**.

Chapter 3

Unilateral renal agenesis: a systematic review on associated anomalies and renal injury

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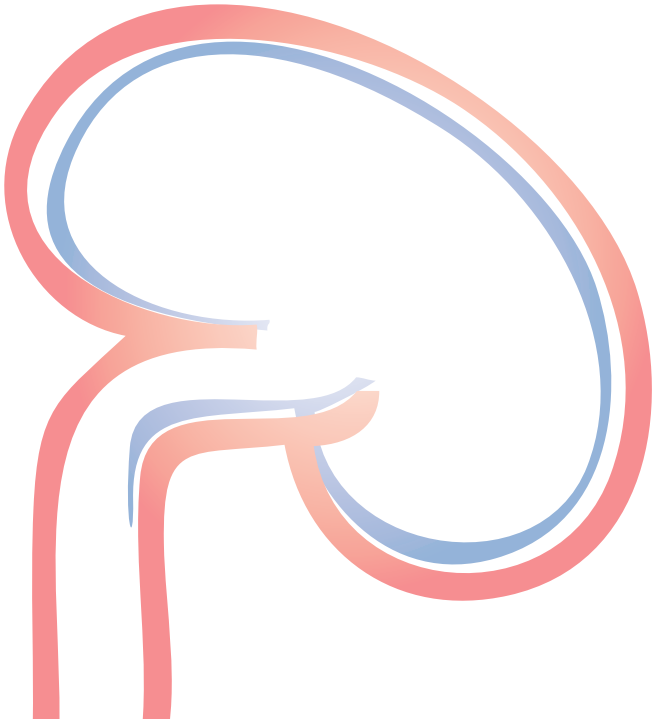

ABSTRACT

Background: Unilateral renal agenesis (URA) is associated with other congenital anomalies of the kidney and urinary tract (CAKUT) and extra-renal anomalies. However, the reported prevalences of these anomalies are highly variable. We estimated the prevalence of associated CAKUT and extra-renal anomalies in patients with URA. Furthermore, we determined the prevalence of renal injury in URA patients.

Methods: We conducted a systematic review of English and non-English articles using PubMed and Embase.com. Included studies reported at least one of the following items: incidence of URA, gender, side of URA, prenatal diagnosis, performance of micturating cystourethrogram, associated CAKUT, urinary tract infection or extra-renal anomalies. Studies that described a mean/median glomerular filtration rate (GFR) and proportions of patients with hypertension, micro-albuminuria or a decreased GFR were also included.

Results: Analyses were based on 43 included studies (total number of patients: 2,684, 63% male). The general incidence of URA was 1 in ~2,000. Associated CAKUT were identified in 32% of patients, of which vesicoureteral reflux was most frequently identified (24% of patients). Extra-renal anomalies were found in 31% of patients. Hypertension could be identified in 16% of patients, whereas 21% of patients had micro-albuminuria. Ten percent of patients had a GFR <60 ml/min/1.73m^2.

Conclusions: These aggregate results provide insight in the prevalence of associated anomalies and renal injury in patients with URA. Our systematic review implicates that URA is not a harmless malformation by definition. Therefore, we emphasize the need for clinical follow-up in URA-patients starting at birth.

INTRODUCTION

Unilateral renal agenesis (URA) is defined as the one-sided congenital absence of renal tissue resulting from failure of embryonic kidney formation.[62,63] Human renal development is initiated at the fifth gestational week and is characterized by highly orchestrated interactions between the outgrowing ureteric bud of the mesonephric duct and the metanephric mesenchyme.[7] As a consequence, renal agenesis occurs when the ureteric bud fails to form the ureter, the renal pelvis and the collecting ducts, and the renal mesenchyme to form nephrons.[64]

URA should be discriminated from abnormal or incomplete renal development leading to a non-functioning kidney, as can be identified in a multicystic dysplastic kidney (MCDK) or renal aplasia.[10] With fetal ultrasonography screening routinely performed, clinicians are more often confronted with an apparent diagnosis of URA. Post-natal ultrasound, renal scintigraphy and/or magnetic resonance imaging can further discriminate between MCDK, renal aplasia or renal ectopia (i.e. pelvic kidney or cross-fused kidney).[65] However, it must be noted that the diagnosis "URA" could derive from the spontaneous involution of the MCDK.[66]

URA is often associated with congenital anomalies of the kidney and urinary tract (CAKUT) of the contralateral kidney,[67] such as pelviureteric junction obstruction (PUJO) and vesicoureteral reflux (VUR). Furthermore, URA patients frequently have extra-renal anomalies, such as cardiac, genital or gastrointestinal malformations.[68] Although studies confirm the association of URA with CAKUT and other malformations,[67-69] the prevalences of these anomalies are not well established.

Assessment of the prevalence of associated malformations in URA may be important as it helps clinicians to ascertain a general and renal prognosis for patients with URA. Some authors report that URA is a more or less harmless congenital malformation,[42,70] whereas a recent study demonstrated that 40-50% of adults with URA required dialysis by the age of 30 years.[2] Although reported in a selected series of URA-patients and thereby likely overestimating the true risk of end-stage renal disease, this impaired clinical outcome may be explained by the hyperfiltration hypothesis, which has been described by Brenner et al. more than three decades ago.[25,26,71] Based on animal studies, this hypothesis states that renal mass reduction leads to a vicious cycle of compensatory glomerular hyperfiltration. In the long run, glomerular hyperfiltration may result in renal injury (i.e. hypertension, micro-albuminuria and/or chronic kidney disease). In addition, we have recently shown that associated ipsilateral CAKUT are an independent risk factor in the development of renal injury in children with a solitary functioning kidney (including URA).[11] Thus, it is important for clinicians to be informed about the contralateral urinary tract in URA-patients.

Therefore, we determined the incidence of URA in the general population as well as the prevalence of associated CAKUT, extra-renal anomalies and renal injury in patients with URA by performing a systematic review of the literature.

METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[72] was followed in this systematic review. A review protocol was absent before the start of this study.

Search strategy for identification of studies

PubMed and Embase.com were searched for relevant studies (by R.W. and J.C.F.K.) from inception to 3 May 2012 (PubMed) and 14 June 2012 (Embase.com). The following terms were used (including synonyms and closely related words) as index terms or free-text words: 'unilateral renal agenesis', 'solitary kidney' and 'hereditary renal agenesis'. Case reports and animal studies were excluded as index terms. The search strategies used can be found in Supplementary Table 3.1. In addition, the 'related articles' function in PubMed as well as reference lists from included publications were searched manually for eligible articles. Furthermore, books that published data on URA-cohorts were used when available.

Study selection

Included studies reported at least one of the following items: gender, side of URA, prenatal diagnosis, performance of micturating cystourethrogram (MCUG), associated CAKUT, urinary tract infection or extra-renal anomalies. Studies that described a mean/median glomerular filtration rate (GFR) or proportions of patients with hypertension, micro-albuminuria and a decreased GFR were also included.

To accurately analyse cohort descriptions on URA, only studies with at least 10 subjects were included. In addition, we excluded studies that exclusively described female patients with URA and anomalies of the female tract to prevent bias on gender distribution and side of URA. For a detailed review on this topic, see [73]. Finally, studies that reported data on the incidence of URA in the general population were included separately.

When several articles described data as (part of) the same cohort, only the study with the largest cohort was included. R.W. and J.A.E.v.W. independently assessed the eligibility of studies using an unblinded and standardized manner. Disagreements between reviewers were resolved by consensus.

Data extraction process

R.W. extracted all data by using Review Manager Version 5.1 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). The following outcome measures were extracted: incidence of URA, gender (male : female), side of URA (right : left), study subjects with a prenatal diagnosis, study subjects investigated by MCUG, associated CAKUT [defined as VUR, PUJO, megaureter, posterior urethral valves (PUV), duplex kidney and ureteroceles], urinary tract infection (one or more), proportion of extra-renal anomalies (cardiac, gastrointestinal, musculo-skeletal and miscellaneous). Finally mean/median GFR (ml/min/1.73m^2) and data on renal injury, i.e. proportions of subjects with hypertension, micro-albuminuria and/or a GFR <60 ml/min/1.73m^2, were obtained.

A number of papers reported combined data of congenital solitary functioning kidney, which encompasses URA as well as MCDK. For 23 studies with missing or combined data on one or more of the outcome measures, we contacted the corresponding author by email to obtain additional data. We received a response from 10 (43%) authors of whom 9 (38%) provided missing data. The remaining studies (n=14) were excluded from analysis.

Data analysis

Analyses were performed using Review Manager Version 5.1 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Data on the incidence of URA were analysed separately. As it may be anticipated that a proportion of patients diagnosed with URA in post-natal studies had actually an involuted MCDK or renal aplasia/dysplasia, we differentiated between cohorts based on timing of diagnosis (i.e. pre- versus post-natal).

Furthermore, we calculated a pooled effect size of weighted mean differences for continuous and proportional data, together with an odds ratio (OR) and 95% confidence interval (CI), based on a random-effects model using meta-analysis methods. A random-effects model was chosen *a priori* as we had the impression that variation in the study populations would result in between-study heterogeneity beyond that of sampling variability. The weight (per cent) is based on study size and variation of the data [standard deviation (SD)]. Second, the proportion of males and left sided URA was compared with the expected proportion (50% or 0.5), taking into account the heterogeneity (I^2) between studies.

Finally, a two-tailed $P \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Study selection

The search of PubMed and Embase.com provided a total of 4,267 citations (for a study flow diagram, see Figure 3.1). In addition, two books[74,75] were available to the authors. After adjustment for duplicates 3,360 citations remained. Of these, 3,245 citations were discarded after reading the title and abstract because of clear non-eligibility to our inclusion criteria. Seven additional studies were excluded because the full-text was not available or the paper could not be translated by the authors. We examined the full-text of 108 papers in detail, of which 40 did not meet the inclusion criteria as described. Forty-three studies were included for meta-analysis with a total number of 2,684 subjects. In addition, 33 studies reported data on the incidence of URA in the general population, of which 8 studies were also included in the meta-analysis.

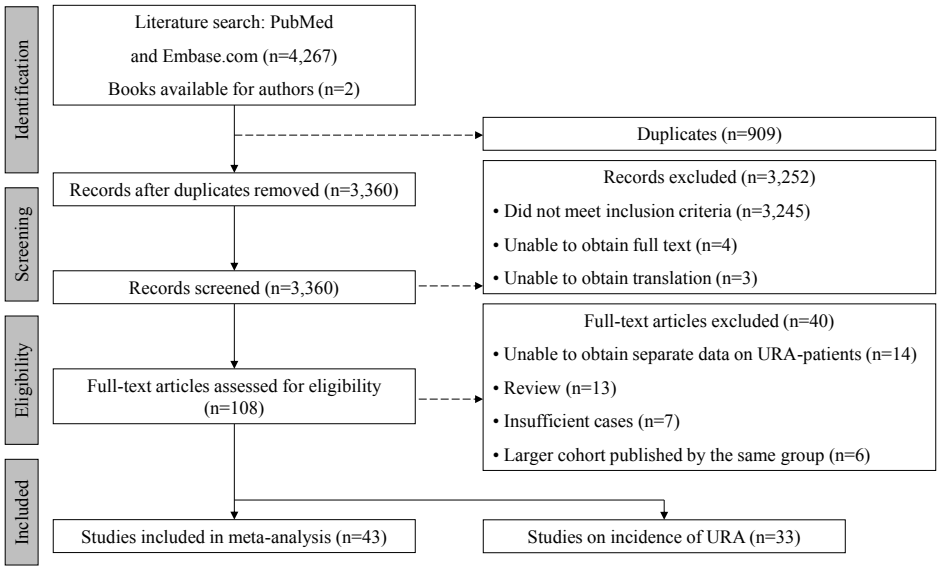

FIGURE 3.1. Flow-diagram of study selection.

Demographics

Table 3.1 presents studies on the incidence of URA. Based on all available data, the general incidence of URA was estimated to be 1 in 2,031, whereas the incidence of URA based on prenatal studies alone (n=10) was 1 in 8,091.

Characteristics of all included studies on the prevalence of CAKUT, extra-renal anomalies and renal injury are shown in Table 3.2. Overall, most subjects with URA were male (63%, OR 1.67, 95% CI 1.49 – 1.87; $P < 0.001$; $I^2 = 59\%$). URA was more often identified

TABLE 3.1. Reported incidences of unilateral renal agenesis.

Study	Year	Country	Timing of diagnosis	Number of patients with URA	Size of population screened	Incidence
Anders[94]	1910	Unknown	Postmortem	51	92,690	1 / 1,817
Eisendrath[95]	1924	USA	Postmortem	85	77,812	1 / 915
Fortune[96]	1927	Unknown	Postmortem	108	139,346	1 / 1,290
Campbell[97]	1928	USA	Postmortem	76	122,320	1 / 1,609
Thompson (cited from[74])	1937	Unknown	Postmortem	32	12,888	1 / 403
Collins[98]	1932	Unknown	Postmortem	572	337,488	1 / 590
Bell[74]	1950	USA	Postmortem	96	59,064	1 / 615
Campbell[75]	1951	USA	Postmortem	88	47,409	1 / 538
Ashley[99]	1960	USA	Postmortem	232	245,000	1 / 1,056
Emanuel[92]	1974	USA	Postnatal	74	132,500	1 / 1,859
Kiprov[100]	1982	USA	Postmortem	7	9200	1 / 1,314
Roodhooft [101]	1984	USA	Adults	2	682	1 / 341
Wilson[102]	1985	USA	Postnatal	90	625,132	1 / 6,946
Helin[103]	1986	Sweden	Prenatal with postnatal confirmation	5	11,986	1 / 2,397
Sheih[104]	1989	China	Postnatal	103	132,686	1 / 1,288
Stoll[79]	1990	France	Postnatal	33	105,374	1 / 3,193
Barakat[105]	1991	USA	Postmortem	25	13,775	1 / 551
Mihara[106]	1992	Japan	Postnatal	3	2,920	1 / 973
Gunn[107]	1995	New Zealand	Prenatal with postnatal confirmation	1	3,856	1 / 3,856
Kim[108]	1996	Korea	Prenatal	2	5,442	1 / 2,721
Harmat[109]	2001	Hungary	Postnatal	66	46,858	1 / 710
Vial[110]	2001	Switzerland	Prenatal with postnatal confirmation	9	38,110	1 / 4,234
Hiraoka[9]	2002	Japan	Postnatal	2	4,000	1 / 2,000
Raboei[111]	2002	Saudi Arabia	Prenatal	8	19,400	1 / 2,425
Yuksel[112]	2004	Turkey	Prenatal	13	13,705	1 / 1,054
Wiesel[19]	2005	Europe-wide	Prenatal with postnatal confirmation	58	709,030	1 / 12,225
Calderon[113]	2006	Colombia	Prenatal and Postnatal	4	30,250	1 / 7,563
McPherson[114]	2007	USA	Postnatal	203	1,129,877	1 / 5,566
Halek[115]	2010	Czech Republic	Postnatal	6	6,088	1 / 1,014
Shipp[116]	2011	USA	Prenatal	2	4,170	1 / 2,085
Zhang[117]	2011	China	Postnatal	22	26,989	1 / 1,227
Caiulo[118]	2012	Italy	Postnatal	11	17,783	1 / 1,617
Melo[119]	2012	Brazil	Prenatal with postnatal confirmation	5	29,653	1 / 5,931

TABLE 3.1. (continued)

Study	Year	Country	Timing of diagnosis	Number of patients with URA	Size of population screened	Incidence
Overall	**1910-2012**			**2,094**	**4,253,483**	**1 / 2,031**
All cohorts with prenatal diagnosis only	**1986-2012**			**107**	**865,602**	**1 / 8,090**

*, The reported patients in this study had prenatal renal aplasia. URA, unilateral renal agenesis.

TABLE 3.2. Characteristics of studies included in meta-analysis.

Study	Year	Age of study population	Subjects	URA side (right:left)	Gender distribution (male:female)	Prenatal diagnosis (%)	Percentage evaluated with MCUG
Collins[98]	1932	Children/adults	512	280 : 209	281 : 231	NR	NR
Soloway[120]	1939	Children/adults	10	5 : 5	5 : 5	NR	NR
Bell[74]	1950	Children/adults	96	43 : 50	52 : 23	NR	NR
Campbell[75]	1951	Children/adults	88	43 : 38	61 : 27	NR	NR
Ashley[99]	1960	Children/adults	232	100 : 132	210 : 22	NR	NR
Rocha-Brito[121]	1969	Children/adults	40	15 : 25	25 : 15	NR	NR
Emanuel[92]	1974	Children	74	27 : 47	52 : 22	NR	30%
Wilson[102]	1985	Children	117	37 : 51	58 : 59	NR	NR
Stojanov[122]	1987	Adults	39	21 : 18	26 : 13	NR	NR
Dinkel[123]	1988	Children	27	NR	17 : 10	NR	NR
Sheih[104]	1989	Children	103	NR	56 : 47	NR	NR
Stoll[79]	1990	Children	33	NR	18 : 15	0%	NR
Janda[91]	1991	Children	23	NR	15 : 8	NR	NR
Argueso[37]	1992	Adults	157	73 : 84	85 : 72	NR	NR
Atiyeh[124]	1993	Children	16	10 : 6	7 : 9	56%	63%
Song[125]	1995	Children	51	22 : 29	27 : 24	22%	86%
De Santo[126]	1997	Adults	21	NR	10 : 11	NR	NR
Palmer[76]	1997	Children	14	8 : 6	NR	NR	100%
Cascio[127]	1999	Children	46	19 : 27	24 : 22	NR	87%
Hill[128]	2000	Children	14	NR	NR	100%	NR
Huang[129]	2001	Children	75	38 : 37	35 : 40	NR	NR
Mei-Zahav[130]	2001	Children	28	NR	13 : 5	NR	NR
Kaneyama[67]	2004	Children	17	NR	NR	18%	100%
Kelm-Kahl[131]	2004	Children	17	NR	NR	NR	NR
Dursun[68]	2005	Children	87	41 : 46	45 : 42	NR	70%
Gonzalez[39]	2005	Adults	33	NR	23 : 10	NR	NR
Guarino[132]	2005	Children	50	21 : 29	37 : 13	NR	100%
De Lucas[133]	2006	Children	47	NR	35 : 12	NR	NR

TABLE 3.2. (continued)

Study	Year	Age of study population	Subjects	URA side (right:left)	Gender distribution (male:female)	Prenatal diagnosis (%)	Percentage evaluated with MCUG
Krzemién[134]	2006	Children	21	8 : 13	NR	33%	90%
Seeman[40]	2006	Children	29	9 : 20	20 : 9	24%	69%
Calisti[135]	2008	Children	55	NR	NR	NR	100%
Hellerstein[136]	2008	Children	45	NR	NR	NR	NR
Vu[42]	2008	Children	20	11 : 9	15 : 5	NR	NR
Sanna-Cherchi[2]	2009	Young adults	71	NR	52 : 19	41%	63%
Spira[137]	2009	Children	22	NR	19 : 3	32%	36%
Wasilewska[93]	2009	Children	65	22 : 43	43 : 22	18%	51%
Gadalean[138]	2010	Adults	20	12 : 8	9 : 11	0%	0%
Wang[139]	2010	Adults	48	23 : 25	30 : 18	NR	NR
Abou-Jaoudé[35]	2011	Children	33	15 : 18	21 : 12	39%	NR
Akl[69]	2011	Children	30	10 : 20	20 : 10	43%	97%
Peco-Antic[44]	2012	Children	14	3 : 11	7 : 7	7%	100%
Taranta-Janusz[90]	2012	Children	38	16 : 22	22 : 16	18%	13%
Westland[13]	2013	Children	99	47 : 52	67 : 32	40%	64%
Overall	**1932-2012**		**2,684**	**979 : 1,080**	**1,542 : 921*** **	**30%**	**67%**

For all studies, the proportions are based on the reported or obtained data. *, For data on renal injury, a subgroup of this cohort[38] was used. **, 12 additional patients were available for analysis. ***, $P < 0.001$ versus the expected proportion of 50%. MCUG, micturating cystourethrogram; NR, not reported and URA, unilateral renal agenesis.

on the left side (52%, OR 1.10, 95% CI 0.97 – 1.25; $I^2 = 0\%$), although this was not different compared to the expected proportion ($P = 0.12$). Based on the papers that reported the timing of diagnosis (n=16), URA was diagnosed prenatally in 173 (30% of reported) patients. MCUG was performed in 549 (67% of reported) patients (n=19 studies).

Prevalence of associated anomalies

Data on associated CAKUT in URA-patients were available from 23 studies with 1,093 patients (Table 3.3). Based on all available data, associated CAKUT were identified in 351 (32%) URA-patients. For every CAKUT-type, the analysis was based on the number of studies that reported the presence (or absence) of that specific item. The most common CAKUT was VUR (24% of reported patients). One study[76] reported patients with URA and VUR only and was excluded from this analysis. Other CAKUT were the presence of a megaureter (7% of reported subjects), PUJO (6%), duplex kidney (3%), ureteroceles (1%) and PUV (1%). In addition, 18 studies reported frequencies of urinary tract infections in patients with URA. Overall, one or more urinary tract infection occurred in 30% of patients (Table 3.3).

TABLE 3.3. Associated anomalies in subjects with unilateral renal agenesis.

	n/N (%)
Associated CAKUT	351/1,093 (32%)
VUR	184/770 (24%)
Megaureter	40/605 (7%)
PUJO	38/615 (6%)
Duplex kidney	22/688 (3%)
Ureterocele	4/555 (1%)
PUV	5/547 (1%)
Urinary tract infection (one or more)	241/813 (30%)
Associated extra-renal anomalies	222/709 (31%)
Female tract anomalies	55/502 (11%)

For every item, the proportion is based on the data available. This explains the differences in denominators between items. CAKUT, congenital anomalies of the kidney and urinary tract; PUJO, pelviureteric junction obstruction; PUV, posterior urethral valves and VUR, vesicoureteral reflux.

Data on extra-renal anomalies could be obtained from 16 studies (Table 3.3). Based on 709 patients, extra-renal anomalies were found in 222 (31%) patients. Data on the type of extra-renal anomalies could be extracted from 12 studies. Of these, gastrointestinal anomalies, cardiac anomalies and musculo-skeletal anomalies were identified in 16%, 14% and 13% of the reported patients, respectively. As a remainder, miscellaneous anomalies (e.g. undescended testis, hypospadia and central nervous system anomalies) were reported in 15% of patients. For studies that reported female tract anomalies [e.g. uterus bicornis, hemivagina, cloaca and Mullerian hypoplasia/aplasia, renal agenesis and cervicothoracic somite dysplasia (MURCS)-syndrome] as part of a larger cohort (n=16), these were described in 55 (11%) female patients.

Prevalence of renal injury

The GFR was reported by 11 studies describing a total number of 437 patients with URA. The calculated pooled mean GFR for all studies was 100 (SD 23) ml/min/1.73m^2.

Data on signs of renal injury, i.e. the presence of hypertension, micro-albuminuria and/or a GFR <60 ml/min/1.73m^2, could be extracted from 23 studies. Of these, 13 (57%) studies reported the age at follow-up (nine pediatric studies, four adult studies). The calculated pooled median age at follow-up was 9.1 (inter-quartile range 9.0 – 10.8) years in pediatric studies and 41.0 (inter-quartile range 40.0 – 57.0) years in adult studies. Based on the available data, hypertension could be identified in 92 (16% of reported) patients, whereas 108 (21% of reported) patients had micro-albuminuria. Finally, 57 (10% of reported) patients had a GFR <60 ml/min/1.73m^2 indicating chronic kidney disease stage ≥3 according to the National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF-K/DOQI) guidelines.[77] In these studies, GFR was determined by

the Schwartz formula (n=6), urinary creatinine clearance (n=4), inulin clearance (n=1) or an unspecified method (n=4).

DISCUSSION

With this systematic review on the prevalence of associated anomalies and renal injury in patients with URA, we have provided an overview of available cohort studies. Our main finding is that one in three patients with URA has additional CAKUT. Furthermore, a large proportion of patients have additional extra-renal anomalies, comprising mostly gastrointestinal, cardiac and musculo-skeletal malformations. Finally, we aggregated data on frequencies of hypertension, micro-albuminuria and an impaired GFR in patients with URA. On the basis of these data, we conclude that one in ~five patients has at least one marker of renal injury. Although our analysis was based on a subset of patients, these findings indicate that URA implies a potential risk to develop chronic kidney disease.[11]

Furthermore, we estimated that the general incidence of URA is 1 in ~2,000. However, it must be noted that there was a large difference in the incidence calculated from studies with a prenatal diagnosis of URA only (1 in ~8,000). Part of this difference is explained by the low incidence of URA found by Wiesel et al.[19] This large study on prenatal congenital renal malformations in 12 European countries found an overall incidence of 1 in ~12,000. Excluding this study would lead to an incidence of URA of 1 in ~3,000 based on prenatal studies. It is well known that URA can be difficult to diagnose prenatally as an empty renal fossa could be easily missed due to a large adrenal gland or mistaken for renal aplasia/dysplasia.[63] The increasing accuracy of prenatal ultrasound screening has contributed to the increase in the overall detection of CAKUT in the last decade.[78] Another explanation for the difference between prenatal and post-natal incidences may be found in studies that estimated incidences based on autopsies performed before the era of prenatal ultrasonography. It may be anticipated that part of these patients in fact had an involuted MCDK[66] or renal aplasia/dysplasia[9] instead of "true" URA, leading to overestimation of the URA incidence. As a consequence of the variability between studies, Robson et al. reported an overall incidence of URA of 1 in 500–3,200.[62] According to our results, URA appears not to be more frequent than 1 in 2,000. With prenatal ultrasonography now routinely available in the majority of countries, we expect the true incidence of URA to become clearer in the coming decade.

In our systematic review, URA was more often identified in males and to be more often located on the left side. In contrast to the well-described male predominance in CAKUT,[79] differences in side were not statistically significant to when URA was expected to occur randomly (i.e. 50% right versus 50% left).

CAKUT account for 34-59% of pediatric and 7% of adult end-stage renal disease worldwide, and has an important impact on renal survival in childhood.[80-82] Based on our analysis, one in every three patients with URA has additional CAKUT, mostly VUR (one in approximately four of reported patients). Unfortunately, we were unable to discriminate between severe VUR (i.e. grade IV-V) and low-grade VUR (grade I-II). Low-grade VUR may be self-limiting and not harmful, whereas severe VUR might impair renal function due to subsequent frequent urinary tract infections.[83] Therefore, we feel that it is important to be informed about high-grade VUR in these children. This could be done by MCUG, which was not routinely performed in most included studies. Furthermore, non-invasive methods to detect VUR have been increasingly described.[84] One promising method is reported by Ismaili et al.,[85] who showed that a normal renal ultrasound rules out high-grade VUR. However, as the validity of this method still needs to be confirmed in larger cohorts,[86] we feel that detecting high-grade VUR in a child with URA requires an individualized approach.

Of the other identified CAKUT, PUJO and especially PUV could severely compromise renal function. A duplex kidney, megaureter and ureteroceles indicate an increased risk for urinary tract infections and secondary impairment of renal function.[11] In addition, we note that the reported prevalence of CAKUT is somewhat comparable to our previous meta-analysis on unilateral MCDK.[10] On the basis of the high prevalences of CAKUT, we strongly encourage that patients with a congenital solitary functioning kidney are clinically monitored throughout life.[87]

Patients with URA also display high proportions of extra-renal anomalies, which could further influence clinical outcome. Genetic factors seem to play a significant role in the development of URA,[12] especially when URA is part of a more or less defined syndrome. Mutations in genes such as *HNF1β*, *PAX2*, *SALL1*, *WT1*, *SIX1* and *EYA1* have all been shown to cause some of these rare syndromes.[7,88] Nevertheless, many of the underlying genes have not yet been identified. One important example of an association without a genetic culprit is VATER association, in which URA is often one of the identified malformations. Although the etiology of VATER association has been identified in a small fraction of patients, future genetic studies can be expected to identify more genetic factors.[89] Not only do these studies provide insight into the primary molecular defects underlying CAKUT, and specifically URA, but they may also contribute to ascertain the prognosis of patients with these specific genetic defects.

Based on 437 patients from 11 studies, the pooled mean GFR was within normal range. Nevertheless, we identified hypertension, micro-albuminuria and renal functional impairment in 16%, 21% and 10% of URA-patients, respectively. This proportion of renal injury might result from glomerular hyperfiltration.[26] However, until now, it is not possible to confirm the hyperfiltration hypothesis in humans due to methodological limitations.[5] Unfortunately, we were unable to calculate the mean age at which renal in-

jury first presented in all URA-patients because the included studies did not provide this data. However, it is noteworthy that our analyses on renal injury mostly consisted of pediatric studies.[13,35,40,42,44,69,90-93] Furthermore, we have recently shown that the median presenting age of renal injury in a large cohort of children with a congenital solitary kidney was ~15 years.[13] According to the hyperfiltration hypothesis, it could be anticipated that adult patients with URA show even higher proportions of renal injury. However, we have not systematically graded the quality of evidence of the included observational studies. This implies that the proportions of renal injury might be influenced by selection bias since the majority of the included studies were performed at a university medical centre (81%, data not shown). Moreover, possible publication bias must be taken into account when interpreting our results. Despite these limitations, our systematic review provide an important explanation for the reported impaired renal outcome of young adults with a congenital solitary kidney.[2] In this elegant study, Sanna-Cherchi et al.[2] demonstrated that adults with URA show a high frequency of end-stage renal disease at 30 years of age, indicating that URA should not be considered a harmless malformation. However, as these results have been described in a selected cohort of patients,[49] generalization of these findings to all URA patients would not be appropriate. Therefore, longitudinal cohort-studies are highly needed to define the clinical outcome of URA-patients. Until a definitive prognosis has been established, we recommend long-term clinical follow-up of blood pressure, urinalysis and GFR in patients with URA.

Our systematic review has several other limitations. The main limitation was the high variability between studies in size of the reported cohorts and the reported prevalences of CAKUT, associated extra-renal anomalies and signs of renal injury. Many studies contained limited data or presented combined results for patients with URA and MCDK or patients with URA and bilateral renal agenesis. In all these studies, we have contacted the corresponding author for additional information. However, missing data from 14 studies could not be obtained. Therefore, we exclusively extracted data for all outcome measures when stratified for URA-patients. As mentioned previously, we could not rule out the influence of selection bias and publication bias on our results. Possible selection bias could also be introduced by studies that have been performed before the era of routine prenatal ultrasonography, since patients with a prenatal diagnosis could be considered as the best surrogate marker of an unselected group of URA-subjects. Unfortunately, few studies described data on associated anomalies or renal injury in 100% of individuals with a prenatal diagnosis of URA. Nevertheless, with the era of routine prenatal ultrasonography, these data should become available in the future.

In conclusion, our systematic review of 43 cohorts describing over 2,600 patients with URA provides insight into the incidence of URA and the demographic characteristics of these patients. Furthermore, we show that one in three patients have associated CAKUT, which are mainly VUR, a megaureter and PUJO. In addition to CAKUT, many

URA-patients have extra-renal anomalies, such as gastrointestinal, cardiac or musculoskeletal malformations. Finally, we demonstrate that URA is not without potential risks as a subset of patients develops hypertension, micro-albuminuria or chronic kidney disease. Until longitudinal cohort studies define the clinical outcome of URA, we emphasize the need for regular follow-up in URA-patients.

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SUPPLEMENTARY TABLE 3.1. Search strategies for PubMed and Embase.com.

PubMed 3 May 2012

MH = MeSH, Medical Subject Headings
[tiab] = words in title or abstract

Search	Query	Items found
#4	#3 NOT (animals [mh] NOT humans [mh])	1,848
#3	#1 NOT #2	2,081
#2	"Case Reports" [Publication Type]	1,566,659
#1	"Hereditary renal agenesis" [Supplementary Concept] OR single kidney*[tiab] OR absent kidney*[tiab] OR solitary functioning kidney*[tiab] OR solitary kidney*[tiab] OR unilateral renal agenesis[tiab] OR hereditary renal agenesis[tiab] OR unilateral renal aplasia[tiab] OR hereditary renal aplasia[tiab] OR aplastic kidney*[tiab]	2,917

Embase.com 14 June 2012

/exp = thesaurus terms exploded
NEAR/ = terms within a number of places next to each other
:ab,ti = words in title or abstract
[embase]/lim = limit to items indexed by Embase, excluding items unique from Medline

Search	Query	Items found
#6	#4 NOT #3 AND [embase]/lim	2,419
#5	#4 NOT #3	2,788
#4	#1 NOT #2	3,271
#3	'animal'/exp OR 'animal experiment'/exp NOT 'human'/exp	2,798,861
#2	'case report'/exp	1,857,989
#1	'solitary kidney'/exp OR (single NEAR/3 kidney*):ab,ti OR (absent NEAR/3 kidney*):ab,ti OR ('solitary functioning' NEAR/2 kidney*):ab,ti OR ('unilateral renal' NEAR/2 agenesis):ab,ti OR ('hereditary renal' NEAR/2 agenesis):ab,ti OR ('unilateral renal' NEAR/2 aplasia):ab,ti OR ('hereditary renal' NEAR/2 aplasia):ab,ti OR (aplastic NEAR/3 kidney*):ab,ti	4,286

Chapter 4

Unilateral multicystic dysplastic kidney: a meta-analysis of observational studies on the incidence, associated urinary tract malformations and the contralateral kidney

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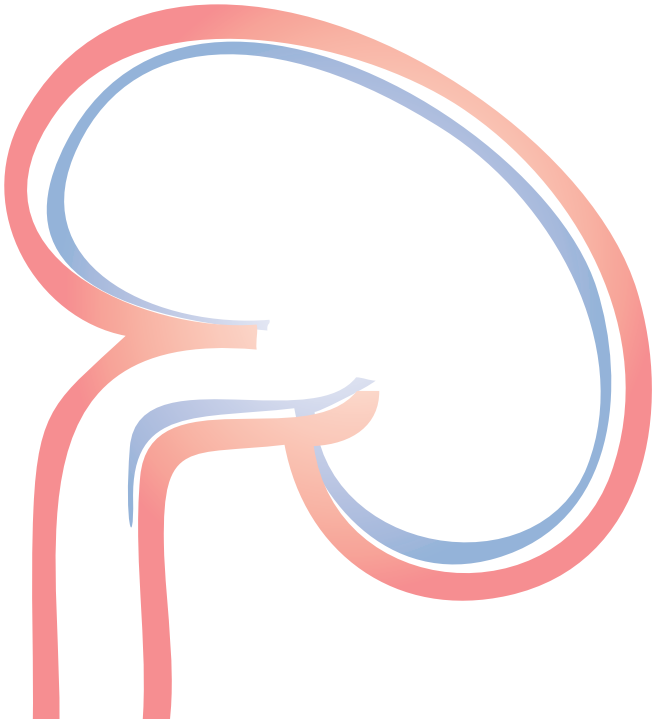

ABSTRACT

Background: Many papers are published on cohorts with unilateral multicystic dysplastic kidney (MCDK) patients but show variable results as to the incidence of associated urinary tract abnormalities. The objective of this study was to describe the status of the urinary tract, including contralateral hypertrophy and malformations, in patients with unilateral MCDK based on a meta-analysis of the literature, taking into account the timing of diagnosis (pre- versus postnatal) as a possible source of bias.

Methods: A systematic review of the scientific literature in English was conducted using PubMed and Embase, identifying all cohorts describing patients with unilateral MCDK. A meta-analysis was performed with the studies that were identified using our reproducible search.

Results: Based on analysis of the data in 19 populations, the overall incidence of unilateral MCDK is 1 in 4,300, with an increasing trend over the years. A total of 67 cohorts with over 3,500 patients with unilateral MCDK were included in the meta-analysis. Fifty-nine percent of patients were male and the MCDKs were significantly more often found on the left side (53.1%). Associated anomalies in the solitary functioning kidney were found in 1 in 3 patients, mainly vesicoureteric reflux (VUR, in 19.7%) and pelviureteric junction obstruction (4.8%). In patients with VUR, 40% have severe contralateral VUR, defined as grade III-V. Contralateral hypertrophy, present in 77% of patients after a follow-up of at least 10 years, showed a trend to be less pronounced in patients with VUR. Timing of the diagnosis of MCDK did not essentially influence the results.

Conclusions: These aggregate results provide insight into the incidence, demographic data and associated anomalies in patients with unilateral MCDK. One in three patients with unilateral MCDK show anomalies in the contralateral solitary functioning kidney. However, studies into the long-term consequences of these anomalies are scarce.

INTRODUCTION

A multicystic dysplastic kidney (MCDK) is a form of renal dysplasia that leads to a non-functioning organ due to abnormal and incomplete kidney development. The first description of an MCDK at autopsy has been in 1836, and the first description of a MCDK removed at surgery was reported a century later.[140] In 1955, MCDK was identified as a separate entity, distinct from polycystic kidneys, which it was generally clustered with until that time.[141] Edith Potter, in her book *Normal and Abnormal Development of the Kidney*,[142] suggested that primary failure of nephron induction was the underlying mechanism leading to MCDKs, resulting in incompletely branched ducts surrounded by connective tissue, which contains undifferentiated and metaplastic cells such as cartilage- and smooth muscle-like cells. Even though no nephrogenic zone at any stage of nephrogenesis, and hence complete absence of nephrons, was described by Potter, MCDKs sometimes do contain some functional renal tissue[143] with recognizable glomeruli and proximal tubules.[144-149] Alternatively, the disruption of normal nephrogenesis could, at least in part, be explained by an impaired fetal urine flow early in development, which is consistent with the general finding of non-patent or atretic ureters attached to MCDKs (for a review[150,151]).

Bilateral MCDK leads to absent fetal and neonatal renal function with associated pulmonary hypoplasia and is therefore generally considered incompatible with extrauterine life.[152] However, unilateral MCDK is a condition that does not lead to any complaints *per se*, except for potential mechanical problems due to a large abdominal mass in rare cases.[153] Before the era of prenatal ultrasound screening, this condition was mainly diagnosed in patients that underwent ultrasound assessment of the renal tract for another reason like a urinary tract infection or a palpable abdominal mass. As many MCDKs are known to show spontaneous involution, even before birth, a significant proportion of patients diagnosed in the era before antenatal ultrasound screening with unilateral renal agenesis (based on the absence of a single kidney) may actually have had a completely regressed MCDK.[9,154,155] This may explain part of the differences in the incidence of unilateral MCDK that have been reported (Table 4.1).[9,19,78,103,104,107-111,156-164] Based on the available ultrasound data, the general incidence can be estimated to be around 1 in 4,300.

MCDK has been described to be associated with general dysmorphologies and contralateral urinary tract abnormalities, like vesicoureteric reflux (VUR) and pelviureteric junction obstruction (PUJO). However, these associated anomalies have been reported in highly variable frequencies. A likely factor in this variation may be the timing of diagnosis of the MCDK (i.e. pre- versus postnatally). It may be anticipated that before the introduction of prenatal ultrasound screening, patients presenting with MCDK were the ones that had clinically relevant associated anomalies. This makes it important to

TABLE 4.1. Reported incidences of unilateral multicystic dysplastic kidney.

Source	Year	Country	Age at diagnosis	Number of patients with unilateral MCDK	Size of population screened	Incidence
Helin[103]	1986	Sweden	Prenatal with postnatal confirmation	7	11,986	1 / 1,712
Gordon[156]	1988	UK	Prenatal with postnatal confirmation	10	43,175	1 / 4,318
Evans[157]	1989	Canada	NR	14	83,893	1 / 5,992
Sheih[104]	1989	China	6-12 years	21	132,686	1 / 6,318
Al-Khaldi[158]	1994	UK	Prenatal with postnatal confirmation	14	43,419	1 / 3,101
Gloor[159]	1995	USA	Prenatal with postnatal confirmation	11	26,770	1 / 2,434
Gunn[107]	1995	New Zealand	Prenatal with postnatal confirmation	8	3,856	1 / 482
Kim[108]	1996	Korea	Prenatal	5	5,442	1 / 1,088
Liebeschuetz[160]	1997	UK	Prenatal with postnatal confirmation	14	33,537	1 / 2,395
Dillon[161]	1998	UK	Prenatal with postnatal confirmation	10	25,382	1 / 2,538
James[162]	1998	UK	Prenatal with postnatal confirmation	22	105,542	1 / 4,797
Kessler[163]	1998	Israel	Various ages	23	NR	1 / 3,310
Harmat[109]	2001	Hungary	Postnatal	13	46,858	1 / 3,604
Vial[110]	2001	Switzerland	Prenatal with postnatal confirmation	23	38,110	1 / 1,657
Hiraoka[9]	2002	Japan	Neonatal	1	4,000	1 / 4,000
Raboei[111]	2002	Saudi Arabia	Prenatal	21	19,400	1 / 924
Ylinen[164]	2002	Finland	Prenatal with postnatal confirmation	51	209,125	1 / 4,100
Wiesel[19]	2005	Europe-wide	Prenatal with postnatal confirmation	105	709,030	1 / 6,753
Mallik[78]	2008	UK	Prenatal with postnatal confirmation	21	46,060	1 / 2,193
Overall*				**371**	**1,588,271**	**1 / 4,281**
All cohorts with postnatal ultrasound confirmation of prenatal diagnosis of MCDK				**296**	**1,295,992**	**1 / 4,378**

*, excluding the article by Kessler et al.[163] as the size of the screened population was not provided. MCDK, multicystic dysplastic kidney and NR, not reported.

differentiate between cohorts that are defined by prenatal screening and cohorts that are based on patients with MCDK that presented with clinical symptoms.

In this paper, we set out to describe the status of the urinary tract, including contralateral hypertrophy and malformations, in patients with unilateral MCDK based on a meta-analysis of the literature, taking into account the timing of diagnosis.

METHODS

Search strategy

A PubMed search was conducted for articles published from January 1966 onwards that contained the keywords "multicystic dysplastic kidney" or "multicystic kidney dysplasia" and/or were labelled with the Medical Subject Heading (MeSH) "multicystic dysplastic kidney" (total hits 373, 2 June 2008). An Embase search was conducted with the search strategy 'multicystic' and ('dysplastic' or 'dysplasia'/exp or 'dysplasia') and ('kidney'/exp or 'kidney'), resulting in 714 hits (2 June 2008). In addition, the 'related articles' function in PubMed was used from articles that were considered for inclusion. Also, reference lists from included publications were searched manually.

Selection of articles

All cohort studies describing the pre- and/or postnatal characteristics of patients with unilateral MCDK were of interest. Title and/or abstract of all articles identified were screened by one of the authors (MFS), and relevant original studies were read in full. Case-reports were specifically excluded from the meta-analysis, as were abstracts only, and articles in non-English languages as this prevented us from accurate analysis of the cohort description. When several articles described (part of) the same cohort, only the study with the most accurate description of the largest cohort (generally being the latest publication) was included. In total, 72 articles were considered for the meta-analysis. Papers excluding part of the cohort on the basis of concomitant anomalies were excluded from our analysis.

Data abstraction

Data on timing of diagnosis (prenatal or postnatal) and the reason for the postnatal investigation were extracted, together with, when specified, the number of patients with complete prenatal ultrasonic involution of the MCDK, and the number of patients with any activity on postnatal renography at the site of the MCDK. Also, the patients' gender and side of the MCDK were obtained. From the retrieved cohorts, we intended to only analyse the children who were diagnosed with unilateral MCDK. However, as some papers reported genders for the unilateral and bilateral MCDK patients combined, data

are presented for all studies together and for studies with only unilateral MCDK patients separately. Based on the classification of the original paper, the number and/or proportion of patients classified as having any urinary tract abnormality (either structural or functional), and the number and/or proportion of patients classified as having extra-urinary tract abnormalities were noted (labelled as associated abnormalities).

The number of patients evaluated with a micturating cystourethrogram (MCUG) was extracted from the papers, and the percentage of patients evaluated with a MCUG was calculated. It can be anticipated that cohorts in which only a selection of patients were assessed with an MCUG are biased, as patients may have been selected that showed clinical reasons to suspect abnormalities. In order to minimize the effect of possible selection of patients that showed clinical abnormalities, a separate analysis of the MCUG results was performed in cohorts where at least 95% of patients were evaluated with a MCUG. VUR was classified as present or absent, and, when present, as ipsilateral (i.e. at the side of the MCDK), contralateral, or bilateral. When available, the grading of VUR according to the International Reflux Study Committee classification was noted.[165] As some papers showed the number of patients per cluster of VUR grades, the clustering as used by the International Reflux Study in Children was adapted, classifying grades III-V as 'severe.'[166]

In each cohort, the number of patients with specific urinary tract abnormalities based on ultrasound and/or renography were noted, and classified as PUJO, ureterovesical junction obstruction, non-obstructive megaureter, ureterocele, posterior urethral valves (PUV), horseshoe kidney, or miscellaneous. When noted, the number of patients with contralateral renal hypertrophy (renal length ≥95th percentile based on the centile chart used in the specific paper) was obtained, as well as differences in renal size between patients with and without contralateral VUR.

Analysis

With these data, a cumulative meta-analysis was performed. Since not all items were reported in all publications, each item is presented as the number of patients in which that item was present divided by the total number of patients in the cohorts that presented data on that specific item. For the associated urinary tract abnormalities, the denominator is based on the number of patients that were evaluated with ultrasound in the cohorts that presented data on this. As a consequence of the highly variable presentation of items amongst the cohorts, the total number presented as denominator in Tables 4.2-4 is different for nearly every item.

Comparison of two proportions of categorical data was done by the chi-square test. As some data indicates that the presence of VUR may influence the size of the solitary functioning kidney opposite the MCDK,[167,168] data from studies comparing renal size between patients with and without VUR,[85,167,168] was combined, and analysed using Review

Manager (RevMan) version 4.2 for Windows (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2003). This enables the calculation of a pooled effect size of weighted mean differences for continuous data together with a 95% confidence interval (95% CI), based on a random-effects model. A random-effects model was chosen *a priori* as we had the impression that variation in the study populations would result in between-study heterogeneity beyond that of sampling variability. The weight (%) is based on study size and variation of the data (standard deviation). Statistical differences were considered significant if $P < 0.05$ (two-tailed).

RESULTS

Characteristics of the cohorts included, including a meta-analysis, are presented in Table 4.2. Four articles excluded part of the cohort based on contralateral urinary tract anomalies, like hydronephrosis or abnormal renal position[169] or the complexity of cases,[170-172] and for another paper, data of patients with MCDK could not be separated from other diagnoses.[173] These cohorts were therefore excluded from our analysis. Other cohorts excluded patients in which not all diagnostic scans were performed[174-176] or that underwent nephrectomy;[177] the demographic data of these cohorts were included in the analysis. Three papers reported results from autopsies, and did not provide any data on the function of the urinary system like VUR.[178-180] Nevertheless, the characteristics were included in Table 4.2. Presentation of the data from 11 cohorts included patients with bilateral MCDK.[146,158,178,181-188] In order to analyse the demographic data of subjects with unilateral MCDK separately, Table 4.2 also shows the obtained data from all patients with unilateral MCDK only.

Overall, the majority of subjects with a unilateral MCDK were male (59.2%, $P < 0.0001$ compared with the expected 51%,[189] and these MCDKs were found on the left side in just over half (53.1%, $P < 0.02$ when compared with the expected 50%). Based on the papers that reported the timing of diagnosis, 19.2% of patients were diagnosed with MCDK postnatally. Indications for ultrasound evaluation of the abdomen and/or urinary tract were an abdominal mass in 64.2%, a urinary tract infection in 7.1%, and various in the rest.

Five studies reported the number of patients that had shown a (sign of) MCDK on prenatal screening but showed no visible renal tissue on the first postnatal ultrasound, thereby showing complete prenatal involution of MCDK.[186,190-193] Combining the data from these five studies, 21 out of 389 (5.4%) individuals showed complete prenatal involution of the MCDK. MCDKs are occasionally reported to show activity on postnatal renography even though histology is consistent with MCDK[4]; seven of the included co-

TABLE 4.2. Characteristics of included studies.

Source	Year	No. of subjects	MCDK side (right : left)	Gender distribution (male : female)	Diagnosis (prenatal : postnatal)	Percentage of unilateral cases evaluated with MCUG	No. (n/N) of nephrectomies
Pathak[178]	1964	21*	9 : 11	15 : 6	0 : 21	NR	9/12
Greene[181]	1971	38*	NR	23 : 15	NR	NR	15/30
Risdon[179]	1971	14	5 : 9	7 : 7	NR	NR	14/14
Gipson[216]	1976	22	9 : 13	11 : 11	0 : 22	NR	22/22
De Klerk[182]	1977	29*	9 : 17	19 : 10	0 : 29	24%	26/26
Walker[217]	1978	11	6 : 5	7 : 4	0 : 11	NR	8/11
Stuck[194]	1982	15	7 : 8	NR	1 : 14	NR	9/15
Kleiner[218]	1986	22	7 : 15	NR	22 : 0 ‡	NR	0/18
Pedicelli[219]	1986	9	5 : 4	5 : 4	3 : 6	NR	1/9
Avni[183]	1987	13*	NR	NR	13 : 0 ‡	NR	NR
Bachmann[220]	1988	11	NR	6 : 5	4 : 7	82%	3/11
Gordon[156]	1988	23	7 : 16	13 : 10	18 : 5	NR	7/21
Vinocur[144]	1988	30	12 : 18	17 : 13	5 : 25	50%	9/30
Kullendorff[221]	1990	29	13 : 16	21 : 8	17 : 12	59%	26/29
Atiyeh[222]	1992	56	28 : 28	25 : 31	22 : 34	88%	NR
Orejas[184]	1992	24*	11 : 12	18 : 6	16 : 8	50%	11/23
Rickwood[185]	1992	44*	22 : 21	32 : 12	44 : 0 ‡	NR	5/43
Akl[223]	1993	24	6 : 18	11 : 13	17 : 7	42%	NR
Chang[224]	1993	12	2 : 10	7 : 5	5 : 7	8%	10/12
Flack[225]	1993	29	15 : 14	19 : 10	22 : 7	97%	NR
Strife[174]	1993	48	21 : 27	26 : 22	28 : 20	92%	5/48
Wacksman[226]	1993	441	198 : 233 #	250 : 191	288 : 153	15%	181/441
Al-Khaldi[158]	1994	44*	15 : 15	27 : 17	30 : 0 ‡	100%	2/44
Mandell[195]	1994	30	14 : 16	20 : 10	30 : 0 ‡	87%	NR
Sapin[146]	1994	60*	26 : 33	40 : 20	54 : 6	Few	35/59
Gloor[159]	1995	11	4 : 7	9 : 2	11 : 0 ‡	64%	1/11
Gough[196]	1995	62	23 : 39	41 : 21	62 : 0 ‡	100%	37/62
Han[227]	1995	11	4 : 7	8 : 3	7 : 4	18%	9/11
Kaneko[228]	1995	7	4 : 3	2 : 5	5 : 2	100%	6/7
Selzman[229]	1995	65	28 : 37	37 : 28	57 : 8	100%	NR
Karmazyn[176]	1997	68#	NR	35 : 24	NR	87%	NR
Rottenberg[175]	1997	66	39 : 28	NR	60 : 6	100%	14/55
John[199]	1998	35	14 : 21	20 : 15	35 : 0 ‡	100%	6/33
Kessler[163]	1998	23	8 : 15	16 : 7	18 : 5	87%	4/23
Perez[230]	1998	49	NR	32 : 17	48 : 1	90%	12/49

TABLE 4.2 (Continued)

Source	Year	No. of subjects	MCDK side (right : left)	Gender distribution (male : female)	Diagnosis (prenatal : postnatal)	Percentage of unilateral cases evaluated with MCUG	No. (n/N) of nephrectomies
Rudnik-Schoneborn[200]	1998	204	111 : 93	108 : 96	134 : 70	42%	40/204
White[231]	1998	33	NR	NR	NR	100%	10/33
Lazebnik[186]	1999	102*	39 : 39	72 : 30	102 : 0 ‡	24%	NR
Feldenberg[187]	2000	35*	9 : 19	28 : 7	NR	23%	NR
Sukthankar[190]	2000	70	28 : 42	31 : 39	70 : 0 ‡	90%	4/70
Fanos[167]	2001	27	14 : 13	17 : 10	27 : 0 ‡	100%	6/27
Oliveira[232]	2001	20	6 : 14	10 : 10	20 : 0 ‡	100%	0/20
Ranke[188]	2001	138*	50 : 75	83 : 52 †	138 : 0 ‡	99%	85/108
Seeman[233]	2001	25	12 : 13	9 : 16	19 : 6	92%	11/25
Abidari[234]	2002	48	22 : 26	30 : 18	NR	100%	NR
Aubertin[191]	2002	73	35 : 38	33 : 32 †	73 : 0 ‡	NR	9/26
Van Eijk[197]	2002	38	18 : 20	24 : 14	38 : 0 ‡	97%	33/35
Metcalfe[143]	2002	54	24 : 30	32 : 22	52 : 2	NR	NR
Eckoldt[235]	2003	93	NR	NR	93 : 0 ‡	95%	51/93
Okada[236]	2003	10	3 : 7	3 : 7	10 : 0 ‡	100%	0/10
Tilemis[237]	2003	41	19 : 22	28 : 13	25 : 16	78%	21/41
Kaneyama[67]	2004	30	NR	NR	22 : 8	100%	NR
Kuwertz-Broeking[238]	2004	97	55 : 42	60 : 37	82 : 15	92%	17/97
Miller[168]	2004	75	48 : 27	44 : 31	52 : 23	100%	25/75
Ylinen[239]	2004	48	20 : 28	26 : 22	37 : 11	NR	32/48
Alconcher[192]	2005	31	9 : 22	17 : 14	31 : 0 ‡	42%	4/31
Al Ghwery[240]	2005	35	18: 17	18 : 17	35 : 0 ‡	100%	0/35
Damen-Elias[198]	2005	100	53 : 47	58 : 41 †	100 : 0 ‡	83%	79/87
Guarino[132]	2005	62	31 : 31	40 : 22	NR	100%	NR
Ismaili[85]	2005	76	35 : 41	44 : 32	76 : 0 ‡	100%	NR
Rahman[213]	2005	69	30 : 39	39 : 30	46 : 23	100%	8/69
Aslam[193]	2006	202	99 : 103	NR	202 : 0 ‡	71%	11/202
Kakkar[180]	2006	27	NR	NR	NR	NR	NR
Krzemien[134]	2006	17	8 : 9	NR	10 : 7	100%	6/17
Merrot[214]	2006	93	49 : 44	52 : 41	93 : 0 ‡	100%	NR
Onal[177]	2006	61	33 : 28	43 : 18	49 : 12	100%	4/72
Vu[42]	2007	36	18 : 18	23 : 13	NR	NR	4/36

TABLE 4.2 (Continued)

Source	Year	No. of subjects	MCDK side (right : left)	Gender distribution (male : female)	Diagnosis (prenatal : postnatal)	Percentage of unilateral cases evaluated with MCUG	No. (n/N) of nephrectomies
Overall		**3,557***	**1,467 : 1,663**	**1,791 : 1,236**	**2,581 : 613**		**947/ 2,630**
			46.9% : 53.1%	**59.2% : 40.8%**	**80.8% : 19.2%**		**36.0%**
Overall, excluding all bilateral cases		**3,009**		**1,434 : 1,061**			
				57.5% : 42.5%			

*, some patients were diagnosed with bilateral MCDK. #, data of some patients were not available. †, the gender of some patients was unknown, for instance due to a termination of the pregnancy. ‡, included in sub-group analysis of cohorts with 100% prenatal diagnosis. MCDK, multicystic dysplastic kidney; MCUG, micturating cystourethrogram and NR, not reported.

horts reported that some patients (in total 27) showed activity on a postnatal renogram at the site of the MCDK (range of activity 1-18%).[143,174,194-198]

Data on contralateral renal hypertrophy at the first ultrasound soon after birth were reported by two papers, and showed that hypertrophy was present in 12 out of 26 (46.2%)[170] and 8 out of 33 (24.2%)[199] patients. Four papers presented data on compensatory renal hypertrophy after a follow up of at least 10 years, which showed hypertrophy in 1/3 (33%),[200] 2/2 (100%),[199] 3/5 (60%)[190] and 35/43 (81%)[193] patients (overall 41/53, 77%).

Table 4.3 shows the data on associated abnormalities. Overall, 14.9% of patients showed malformations outside the urinary tract, which was similar in the selected cohorts with 100% prenatal diagnosis. Urinary tract malformations were described in 31.3% of patients with unilateral MCDK, which was significantly higher (35.9%, $P < 0.02$) in the prenatally diagnosed cohorts; the majority of these abnormalities consisted of VUR.

In cohorts that described results from MCUGs (n=51), on average 70% of the subjects within the cohort had at least one MCUG performed (Table 4.2). Overall, 19.7% of patients with a unilateral MCDK had VUR (Table 4.4); the mean of the reported incidences was 19.3% (95% CI 15.3-23.3%). This could be classified as severe in 40.5% (i.e. 8.0% of patients with unilateral MCDK show severe VUR). Excluding studies that could potentially have caused bias did not influence these results significantly (17.8% VUR, of which 40.0% graded severe, i.e. 7.1% of patients with unilateral MCDK show severe VUR). Other malformations included PUJO in 4.8% of patients, ureteroceles in 1.3% of patients, horseshoe kidney in 0.60% of patients and PUV in 0.42% of patients (Table 4.3).

A few cohorts reported data on renal size differences between patients with and without VUR.[38-40] One paper showed significant smaller kidneys at birth and at 2 years of

TABLE 4.3. Demographic details of subjects with multicystic dysplastic kidneys.

	All cohorts	Cohorts with 100% prenatal diagnosis only	*P*-value
No. of articles	67	23	-
No. of subjects	3,557	1,369	-
- male*	1,791/3,027 (59.2%)	611/1,027 (59.5%)	NS
- left sided MCDK*	1,663/3,130 (53.1%)	656/1,211 (54.2%)	NS
Associated anomalies*	1,99/1,340 (14.9%)	136/915 (14.9%)	NS
Associated urinary tract abnormalities*	757/2,415 (31.3%)	299/834 (35.9%)	< 0.02
VUR present*	415/2,104 (19.7%)	196/962 (20.4%)	NS
PUJO*	103/2,159 (4.8%)	34/934 (3.6%)	NS
Ureterocele*	29/2,159 (1.3%)	12/934 (1.3%)	NS
Horseshoe kidney*	13/2,159 (0.60%)	4/934 (0.43%)	NS
PUV*	9/2,159 (0.42%)	5/934 (0.54%)	NS

*, data presented as n/N (%). NS, not significant; PUJO, pelviureteric junction obstruction; PUV, posterior urethral valves and VUR, vesicoureteric reflux.

TABLE 4.4. Data on vesicoureteric reflux in subjects with a unilateral multicystic dysplastic kidney.

	All cohorts	Cohorts with MCUG in at least 95% of subjects	Cohorts with MCUG in at least 95% of subjects and 100% prenatal diagnosis of MCDK
No. of papers	67	24	12
No. of subjects	3,557	1,233	671
VUR*	415/2,104 (19.7%)	212/1,164 (18.2%)	109/614 (17.8%)
- contralateral*	267/1,783 (15.0%)	154/1,032 (14.9%)	78/577 (13.5%)
- ipsilateral*	59/1,766 (3.3%)	29/1,032 (2.8%)	25/577 (4.3%)
- bilateral*	42/1,766 (2.4%)	24/1,032 (2.3%)	17/577 (2.9%)
VUR contralateral			
- mild (I-II)*	100/168 (59.5%)	60/107 (56.1%)	27/45 (60.0%)
- severe (III-V)*	68/168 (40.5%)	47/107 (43.9%)	18/45 (40.0%)
- grade I*	21/135 (15.6%)	15/101 (14.9%)	10/45 (22.2%)
- grade II*	54/135 (40.0%)	40/101 (39.6%)	17/45 (37.8%)
- grade III*	32/135 (23.7%)	22/101 (21.8%)	7/45 (15.6%)
- grade IV*	21/135 (15.6%)	17/101 (16.8%)	9/45 (20.0%)
- grade V*	7/135 (5.2%)	7/101 (6.9%)	2/45 (4.4%)

*, data presented as n/N (%). MCDK, multicystic dysplastic kidney; MCUG, micturating cystourethrogram and VUR, vesicoureteric reflux.

age in patients with VUR compared with patients without VUR,[167] whereas the other two reported no significant difference between the groups.[85,168] Combining the data at the age of ~2 years, available from 2 papers,[167,168] the solitary functioning kidney with VUR showed a trend to be smaller than the one without VUR [mean difference –0.88cm (95% CI –1.82 – 0.07 cm, $P = 0.07$)].

DISCUSSION

With this meta-analysis on demographic data and analysis of the contralateral urinary tract in patients with unilateral MCDK, we have provided an overview of the available cohorts that were published in English. Based on the data in Table 4.1, the overall incidence of unilateral MCDK can be estimated to be around 1 in 4,300. The data on complete prenatal involution of MCDKs indicate that a difference can be expected in the incidence between pre- and postnatal diagnosed cohorts. Including only the papers that based the diagnosis of MCDK on combined prenatal and postnatal ultrasound evaluation, the incidence is approximately 1 in 4,400, which is slightly lower than all incidence data together. The fact that the incidence of MCDK may be increasing is also important in the interpretation of our results. Data from consecutive large cohorts in a specific region in the UK showed an incidence of 1 in ~4,800 births in 1984-1988,[162] whereas in the recent cohort (1999-2003) MCDK was present in ~1 in 2,200 births.[78] The overall incidence of urinary tract abnormalities has increased as well in this region, which, according to the authors, is most likely to be secondary to the increasing sensitivity and accuracy of ultrasound screening.[78] Even though the reported sensitivity for the prenatal diagnosis of MCDK is only 53.3% during the period from 1985 to 1996,[201] the doubling in incidence of a gross malformations as MCDK is less likely to be explained by an increase in diagnostic sensitivity alone. Another explanation for the increasing incidence may be found in the increasing incidence of pre-existing diabetes during pregnancy,[202,203] which has been associated with a higher incidence of MCDK.[164] On the other hand, an alternative explanation for the association between diabetes and MCDK can be found in the renal cysts and diabetes syndrome (RCAD syndrome, OMIM #137920), a syndrome based on mutations of the hepatocyte nuclear factor-1beta (HNF-1β).[204] This may explain some of the familial associations that have been described to occur for MCDK.[205-209] Environmental influences, such as maternal antiepileptic drugs,[210] on the occurrence of MCDK have been identified, as well as chromosomal defects[211] and other syndromes than RCAD that are associated with MCDK.[64]

Based on data from the 67 included studies, MCDK is significantly more frequently found on the left side (53.1%). Also, there is a male predominance (59.2% male), which is commonly found with renal tract malformations.[152] In total, seven cohorts reported

on activity on renography at the side of the MCDK in a total of 27 patients. No overall percentage is presented for this number, as most cohorts did not report the number of positive or negative cases on renography, most likely as it was found to be 0. In our opinion, estimation of the overall percentage of MCDKs that show activity on renography is less important than to recognize the fact that some activity on renography does not exclude the diagnosis of MCDK.

Prenatal hypertrophy of the contralateral kidney was found in 24-46% of patients with unilateral MCDK in 2 cohorts included in our analysis. Glazebrook et al.[212] described prenatal hypertrophy in 17 out of 27 (63.0%) patients with congenital solitary functioning kidneys and Hill et al.[128] in 16 out of 36 (44.4%). However, both papers did not report the data for patients with unilateral renal agenesis and unilateral MCDK separately. Whether this hypertrophy is associated with an increase in nephron number remains to be determined. However, this was only 70% of total numbers in 2 kidney controls, which would still leave these patients with a low nephron endowment.

Overall, 1 in ~3 patients with unilateral MCDK has an associated urinary tract malformation, mostly being VUR in 1 in ~5. Of the patients with VUR, about 40% will have severe (grade III-V) VUR. As low grade VUR is more and more recognized to be relatively self-limiting and not harmful, the discussion about the need to perform MCUG in children with urinary tract infections with "normal" renal tracts is ongoing. As 1 in 12-14 children with unilateral MCDK will have severe VUR (Table 4.4), we feel that it is important to be informed about the presence of VUR in children with unilateral MCDK. Whether normal ultrasounds of the solitary kidney can be used to rule out non-low grade VUR and therefore the need for MCUG as suggested by Ismaili et al.,[85] remains to be determined in larger cohorts.

Another contralateral urinary tract malformation is PUJO, which occurs in ~4-5% and may be severe enough to cause acute renal failure.[193,213] Ureteroceles have been described frequently as well in patients with MCDK, but usually show a benign course.[134] A horseshoe kidney was described in 0.6%, which is higher than the estimated incidence of 0.15% in the general population;[214] indeed, several reports on MCDK in horseshoe kidneys have been published, which may show an association between the two conditions (for an overview, see [215]).

Our meta-analysis has several limitations. Most importantly, there was a high variability in the reported incidences of MCDK and the various associated (urinary tract and general) malformations. The high variability in the reported incidences may be explained by the era in which the diagnosis was made (i.e. before the introduction of prenatal ultrasound screening versus after introduction of standard prenatal ultrasound screening). Other possible explanations may be found in the size of the reported cohorts, the introduction of prenatal screening, and adherence to a standardized schema of postnatal investigations once the diagnosis was made. Excluding cohorts that included pa-

tients in whom the diagnosis of MCDK was made postnatally did not basically influence the results. Only a difference in the proportion of associated urinary tract malformations was found, which was higher when the postnatally diagnosed patients were excluded. This was surprising, as we expected that patients diagnosed postnatally would have had a clinical reason to suspect a urinary tract malformation, thereby focussing on a group with a higher incidence of associated anomalies. A possible explanation may be that the reason for the postnatal investigation was a palpable abdominal mass in two-thirds, which is not expected to be influenced by any associated malformations like VUR.

In conclusion, our meta-analysis of 67 cohorts with over 3,500 patients with unilateral MCDK has shown the demographics of this patients cohorts, male and MCDKs on the left side. Analysis of the data in 19 populations showed an overall incidence of unilateral MCDK of 1 in 4,300 with an increasing trend over the years. Associated anomalies in the solitary functioning kidney were found in 1 in 3 patients, mainly VUR and PUJO. Severe contralateral VUR, defined as grade III-V, was found in 1 in every 12-14 patients with unilateral MCDK.

Chapter 5

Renal injury in children with a solitary functioning kidney

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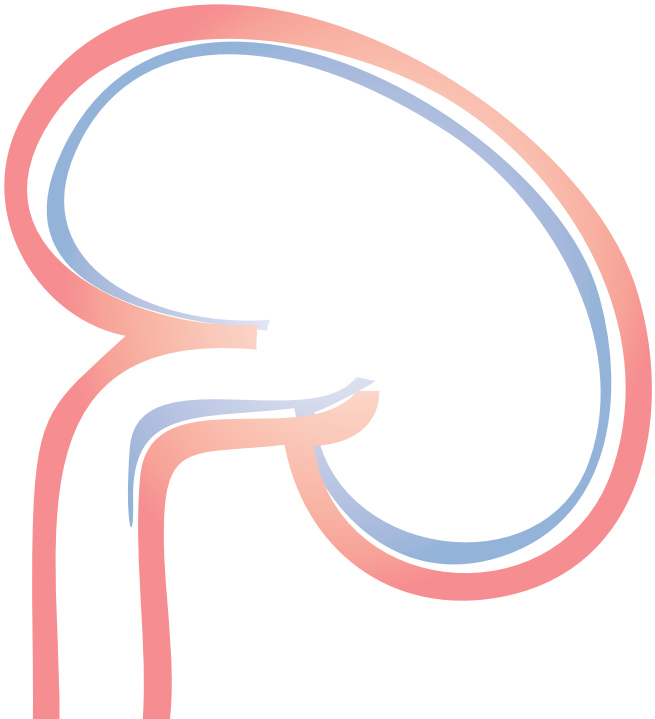


ABSTRACT

Background: Children with a solitary functioning kidney (SFK) have an increased risk of developing hypertension, albuminuria and chronic kidney disease in later life. This renal injury is hypothesized to be caused by glomerular hyperfiltration that follows renal mass reduction in animal studies. Furthermore, children with an SFK show a high incidence of congenital anomalies of the kidney and urinary tract (CAKUT), which could further compromise renal function.

Methods: A retrospective study of renal injury markers was performed in 206 children, divided into groups based on origin of SFK (primary (congenital) SFK [n=116] and secondary SFK [n=90]). Data on ipsilateral CAKUT were stratified separately. For blood pressure, albuminuria and glomerular filtration rate, longitudinal models were additionally developed using generalized estimated equation analysis.

Results: Renal injury, defined as the presence of hypertension and/or albuminuria and/or the use of renoprotective medication, was present in 32% of all children with an SFK at a mean age of 9.5 (SD 5.6) years. Children with ipsilateral CAKUT had higher proportions of renal injury (48.3% versus 24.6%, $P < 0.05$). Furthermore, longitudinal models showed a decrease in glomerular filtration rate in both groups from the beginning of puberty onwards.

Conclusions: This large cohort study demonstrates that renal injury is present in children with an SFK at a young age, whereas our longitudinal models show an increased risk for chronic kidney disease in adulthood. Renal injury is even more pronounced in the presence of ipsilateral CAKUT. Therefore, we underline that clinical follow-up of all children with an SFK is needed.

INTRODUCTION

Children with a solitary functioning kidney (SFK) are at potential risk of developing hypertension, albuminuria and chronic kidney disease in later life.[36,37,39] Several reports have studied the long-term outcome of children with an SFK;[38,40-42,70,130,193] however conclusions remain conflicting.[3] The rationale behind these studies is the 'hyperfiltration hypothesis' described by Brenner et al. in the 1980s.[25,26,71] In their groundbreaking studies using animal models, subtotal renal mass reduction resulted in hypertension, proteinuria and glomerulosclerosis due to glomerular hyperfiltration in remnant nephrons. Moreover, compensatory hypertrophy was found in the remnant kidney.[241] All this sets a perpetuating cycle of nephron loss and, since new nephrons cannot be formed after birth, may result in chronic kidney disease. In human studies, the hyperfiltration hypothesis remains unproven, most probably due to the inability to measure single nephron glomerular filtration rate (GFR) and, more importantly, total nephron number *in vivo*. Nevertheless, a low nephron number is described in deceased patients with hypertension.[31] By definition, an SFK from childhood onwards implies renal mass reduction for a longer period of time, suggesting that children with an SFK have an increased risk for developing hypertension, albuminuria and chronic kidney disease.

An SFK in childhood can be of either primary (congenital) origin (pSFK) or secondary after unilateral nephrectomy (sSFK) due to congenital anomalies of the kidney and urinary tract (CAKUT). Different anomalies underlie a pSFK. Unilateral renal agenesis (URA) involves the complete absence of developing renal tissue and a ureter in fetal life (estimated incidence 1:500-1,000 births[63]). It is suggested that most cases diagnosed as "URA" are actually cases of renal aplasia (i.e. abnormal renal elements that involute), which is not easily detectable on ultrasound.[9] As these entities are not distinguishable in daily clinical practice, both agenesis and aplasia are referred to as URA. In a multicystic dysplastic kidney (MCDK), renal parenchyma shows dysplastic and cystic differentiation with a normally atretic ureter due to abnormal fetal renal development (incidence 1:4,300 births[10]).

Unilateral nephrectomy is performed after severe loss of function due to recurrent urinary tract infection, which can occur with- or without the presence of vesicoureteric reflux (VUR), or obstructive nephropathy. The latter mainly encompasses pelviureteric junction obstruction (PUJO), ureterovesico junction obstruction (UVJO) and posterior urethral valves (PUV) in boys. An sSFK can also be acquired after renal malignancy. This diagnosis often requires subsequent nephrotoxic chemotherapy.

Another factor that may contribute to the increased risk of chronic kidney disease in children with an SFK is the high incidence of associated anomalies in this population.[68] CAKUT are the most important cause of chronic renal disease in childhood,[64] and become increasingly important in adult life.[80] A recent report on renal outcome in

patients with CAKUT demonstrated that approximately ~50% of children with a pSFK required dialysis at 30 years at age.[2] In line with this, our research group showed that 50% of children with a pSFK showed signs of renal injury at the mean age of 8 years.[242]

The KIMONO-study (KIdney of MONofunctional Origin) is designed to study the development of renal injury in children with different origins of SFK. Therefore, we study the presence of hypertension, albuminuria and impaired glomerular filtration rate (GFR) in a large cohort of children with either a pSFK or an sSFK. As the presence of ipsilateral CAKUT in an SFK may imply a more severe clinical course of renal injury, we stratified according to the presence of ipsilateral CAKUT. Finally, in order to study the clinical course of SFK and renal injury into adulthood, we developed a model for blood pressure, albuminuria and GFR related to age.

METHODS

Subjects

All patients with an SFK diagnosed under the age of 18 years, known at the Pediatric Renal Center of the VU University Medical Center were included. In order to avoid possible confounding due to nephrotoxic medication, all children with an sSFK after a renal malignancy were excluded from the study. Part of this cohort was described previously.[242] Diagnosis was confirmed by unilateral absence of (functional) renal tissue on ultrasound and/or on renal scintigraphy. Nephrectomy was generally performed when renal uptake on scintigraphy was <10%.

Ipsilateral CAKUT (i.e. the side of the SFK only) was evaluated by renal ultrasonography and, on indication, through voiding cystourethrogram (n=140, 68%) or renal scintigraphy (n=127, 62%). When a patient had multiple anomalies, the causative and/or most severe CAKUT was selected. Ipsilateral CAKUT also included VUR, which was graded using the system of the International Reflux Study in Children.[165] Clustering of VUR grades was adapted, classifying Grades III-V as 'severe'. Low grade VUR (Grades I-II) is of less clinical importance and is more likely to be missed on renal ultrasound compared to severe VUR.[166] Our retrospective design implicated that voiding cystourethrograms were not performed in all patients. Consequently, this hampers identification of all patients with low grade VUR and therefore only patients with severe VUR were noted as having ipsilateral CAKUT.

The design of this study is shown in Figure 5.1. Patients were divided based on origin of SFK. The pSFK group consisted of (i) patients with URA and (ii) patients with unilateral MCDK. Children with sSFK encompassed (iii) patients with obstructive nephropathy due to PUJO, UVJO, PUV, ureteroceles or a duplex kidney and (iv) patients with a history

of reflux nephropathy and/or renal infection. Subgroups were divided into children with and without ipsilateral CAKUT based on the origin of SFK (Figure 5.1).

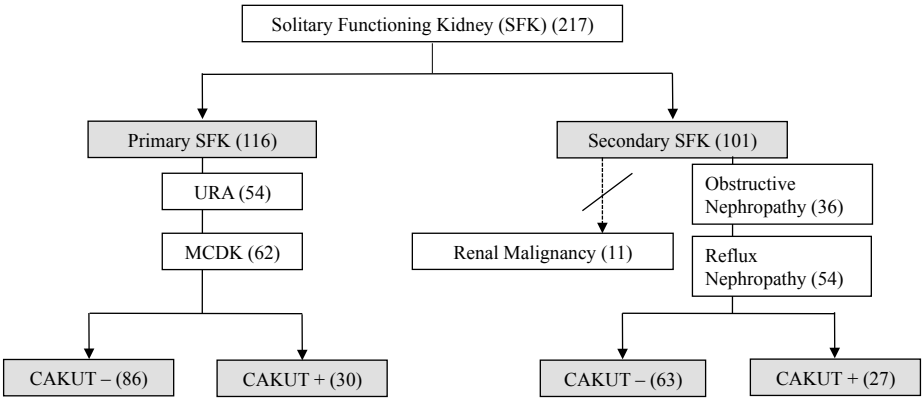


FIGURE 5.1. KIMONO-study design.
Patients (*n*) were divided based on the origin of the solitary functioning kidney. A primary solitary functioning kidney is of congenital origin, and a secondary solitary functioning kidney is acquired after unilateral nephrectomy. Patients with a solitary kidney after a renal malignancy were excluded from analysis. Four diagnosis groups were discriminated. Ipsilateral anomalies of the kidney and urinary tract were stratified for both origins of a solitary functioning kidney. CAKUT, congenital anomalies of the kidney and urinary tract; MCDK, multicystic dysplastic kidney; SFK, solitary functioning kidney and URA, unilateral renal agenesis.

Measurements

Retrospective data were collected by chart review using a standard protocol for testing. Clinical and laboratory data ranged from presentation in our medical centre until most recent follow-up. Data on 689 clinical visits (mean 3.3, range 1-5 visits per patient) were available for analysis. Blood pressure was measured thrice during the same visit with an automated oscillometric method (Dinamap Pro 100; Tefa-Portanje BV, Woerden, The Netherlands), using an appropriately sized cuff. In order to minimize the effect of stress on blood pressure data, the lowest reading of these measurements was used for analysis. Standard deviation scores (SDS) were calculated by using normal values for age, gender and height as previously published.[243] Hypertension was defined as a systolic- and/or diastolic blood pressure ≥2.0 SDS.

Urinary albumin was measured in timed urine collections. (Micro-)albuminuria is expressed in μicrograms per minute and is defined as a urinary albumin excretion ≥20 μg/min.[244]

GFR (ml/min/1.73m^2) was estimated by the Schwartz formula [estimated glomerular filtration rate (eGFR)] corrected for gender and age.[245] Impaired renal function was defined as an eGFR <60 ml/min/1.73m^2.

Some children were treated for hypertension and/or albuminuria with drugs such as angiotensin converting enzyme inhibitors, angiotensin receptor blockers, diuretics, beta blockers and/or calcium antagonists. Since these patients no longer exhibited overt symptoms of renal injury, they were noted as using renoprotective medication. Renal injury was defined as the presence of hypertension and/or albuminuria and/or the use of renoprotective medication.

Renal length (centimeter) was measured by renal ultrasound compared to normal standards of children with two kidneys and expressed as SDS.[246-248] Compensational renal hypertrophy was defined as a renal length ≥2.5 SDS.

Statistical analysis

All data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL., USA). Patient characteristics are presented as mean (SD) for normal distributed parameters and as median (interquartile range, IQR) for non-normally distributed parameters. Subsequent to the retrospective design of this study, results of continuous parameters are based on available data. For proportional variables, missing data were considered to be within normal range. Prior to all analyses, a logistic transformation was performed if a variable was non-normally distributed according to the Shapiro Wilson test. Differences for continuous parameters were hereafter analysed using Student's *t*-test. Proportions in categorical data between groups were analysed with the chi-square test. In all groups, based on the type of SFK and the presence of ipsilateral CAKUT, the longitudinal relation of blood pressure, blood pressure SDS, albuminuria and eGFR was analysed by using separate Generalized Estimated Equation (GEE)-analyses. This analysis takes into account that the same patient was measured repeatedly and utilizes all data available, irrespective of the number of repeated measurements. GEE-analysis is capable of handling irregular time intervals and corrects for the dependency of observations by adding a "within subject correlation structure" to the regression model.[249] An exchangeable correlation structure was used, which means that correlations between subsequent measurements are assumed to be the same, irrespective of the time between measurements. Age (years) and the interaction between groups and age were used as independent variables. Also, higher order terms of these independent variables and their interaction with different groups were included in the analysis. A P-value < 0.05 was considered statistically significant in all analyses.

RESULTS

Patients' characteristics are presented in Table 5.1. Of the 206 patients eligible for analysis, 116 (56%) children had a pSFK and 90 (44%) children had an sSFK. Fifty-four

TABLE 5.1. Patient characteristics by solitary functioning kidney type.

	Primary SFK n=116	Secondary SFK n=90	SFK (Total) n=206
Males (%)	73 (63)	56 (62)	129 (63)
CAKUT (%)	30 (26)	27 (30)	57 (28)
Severe VUR (%)	10 (9)	7 (8)	17 (8)
PUJO (%)	6 (5)	5 (6)	11 (5)
PUV (%)	0 (0)	4 (4)	4 (2)
UVJO (%)	7(6)	3 (3)	10 (5)
Ureteroceles (%)	0 (0)	1 (1)	1 (0)
Duplex system (%)	2 (2)	4 (4)	6 (3)
Hypodysplasia (%)	5 (4)	3 (3)	8 (4)

Results based on data available. A primary solitary functioning kidney is of congenital origin, an SFK is a solitary functioning kidney which is acquired after unilateral nephrectomy. Data presented as count (%). CAKUT, congenital anomalies of the kidney and urinary tract; PUJO, pelviureteric junction obstruction; PUV, posterior urethral valves; SFK, solitary functioning kidney; UVJO, ureterovesico junction obstruction and VUR, vesicoureteric reflux.

patients were diagnosed with URA and 62 patients had a MCDK, whereas 36 patients and 54 patients had an sSFK due to obstructive nephropathy and reflux nephropathy, respectively (Figure 5.1). Ipsilateral CAKUT were identified in 30 (26%) children with a pSFK and 27 (30%) children with an sSFK. In both groups, ipsilateral VUR Grades III–V was found most frequently. Other commonly identified CAKUT were PUJO and UVJO. Ipsilateral CAKUT was more frequently present in children with URA (n=20, 37%) than in children with MCDK (n=10, 16%; $P = 0.010$).

Clinical characteristics at the most recent follow-up are presented in Table 5.2. Overall mean age at follow-up was 9.5 (SD 5.6) years. Mean follow-up time for all children was 6.0 (SD 4.1) years. Mean systolic- and diastolic blood pressure SDS for both origins of SFK were within normal ranges (data not shown). However, hypertension was identified in 17 (15%) children with a pSFK and 10 (11%) children with an sSFK [P = not significant (NS)]. In pSFK, more children diagnosed with URA (n=13, 24%) were hypertensive than children with MCDK (n=4, 6%; $P = 0.01$). Albuminuria was manifested in one of every eight children with an SFK, whereas a large proportion of children were using renoprotective medication at time of follow-up (pSFK: n=21, 18% versus sSFK n=17, 19%; P = NS). Sixty-five (32%) children with an SFK met the criteria for renal injury, 37 (32%) in pSFK and 28 (31%) in sSFK (P = NS). Within the pSFK group, children with URA differed in proportion (n=25, 46%) of renal injury from children with MCDK (n=11, 18%; $P = 0.02$). All children with an eGFR <60 ml/min/1.73m^2 also demonstrated other symptoms of renal injury. Renal hypertrophy was identified in 89 (43%) of cases. Children with renal

TABLE 5.2. Clinical characteristics of children with a solitary functioning kidney.

	Primary SFK			Secondary SFK		
	Total	CAKUT -	CAKUT +	Total	CAKUT -	CAKUT +
N (%)	116	86 (74)	30 (26)	90	60 (67)	30 (33)
Age at follow-up, years (SD)	8.4 (6.5)	8.2 (5.7)	8.8 (5.6)	10.8 (5.5)	10.6 (5.7)	11.0 (4.8)
Follow-up, years (SD)	5.7 (4.1)	5.8 (4.2)	5.5(4.0)	6.3 (4.0)	5.3 (3.6)	8.3 (4.1)*
Renal parameters						
Hypertension (%)	17 (15)	12 (14)	5 (17)	10 (11)	6 (10)	4 (15)
Albuminuria (%)	13 (11)	5 (6)	8 (27)*	11 (12)	6 (10)	5 (19)
Serum creatinine, µmol/l (IQR)	65 (31)	61 (23)	78 (47)*	74 (32)#	74 (33)	83 (37)
eGFR, ml/min/1.73m^2 (IQR)	95 (23)	97 (21)	83 (24)*	94 (19)	94 (15)	94 (27)
eGFR <60 ml/min/1.73m^2 (%)	5 (4)	2 (2)	3 (10)	5 (6)	2 (3)	3 (11)
Renal injury						
Renoprotective medication (%)	21 (18)	9 (11)	12 (40)*	17 (19)	8 (13)	9(33)*
Hypertension and/or albuminuria and/or renoprotective medication (%)	37 (32)	21 (24)	16 (53)*	28 (31)	15 (24)	13 (48)*
and eGFR<60 ml/min/1.73m^2 (%)	5 (4)	2 (2)	3 (10)	5 (6)	2 (3)	3 (11)
Renal hypertrophy						
Renal length ≥2.5 SDS (%)	56 (48)	43 (72)	13 (65)	33 (36)	25 (40)	8 (30)

Data on clinical markers for renal injury based on the origin of the solitary functioning kidney (SFK). In subsequent columns this data is stratified for the presence of ipsilateral congenital anomalies of the kidney and urinary tract (CAKUT) based on origin of SFK. A primary SFK is of congenital origin, a secondary SFK is acquired after unilateral nephrectomy. Data are presented as Count (%), mean (SD) or median (IQR). #, $P < 0.05$ sSFK versus pSFK; *, $P < 0.05$ CAKUT+ versus CAKUT. CAKUT, congenital anomalies of the kidney and urinary tract eGFR, estimated glomerular filtration rate by Schwartz formula; IQR, interquartile range and SDS, standard deviation score.

injury had renal hypertrophy in 38% of cases, whereas children without renal injury had renal hypertrophy in 45% of cases (P = NS).

When both groups were analysed based on origin of SFK and presence of ipsilateral CAKUT, children with a pSFK and ipsilateral CAKUT had a higher proportion of albuminuria ($P = 0.03$) and a significantly lower eGFR ($P < 0.001$) than pSFK children without CAKUT (Table 5.2). Proportions of hypertension were identical between groups. Nevertheless, children with pSFK and CAKUT more often used medication ($P = 0.03$) and showed a higher proportion of renal injury ($P = 0.02$). In line with this, a higher proportion of children with an sSFK and CAKUT used renoprotective medication ($P = 0.02$) and more often demonstrated renal injury ($P = 0.02$) than children without CAKUT.

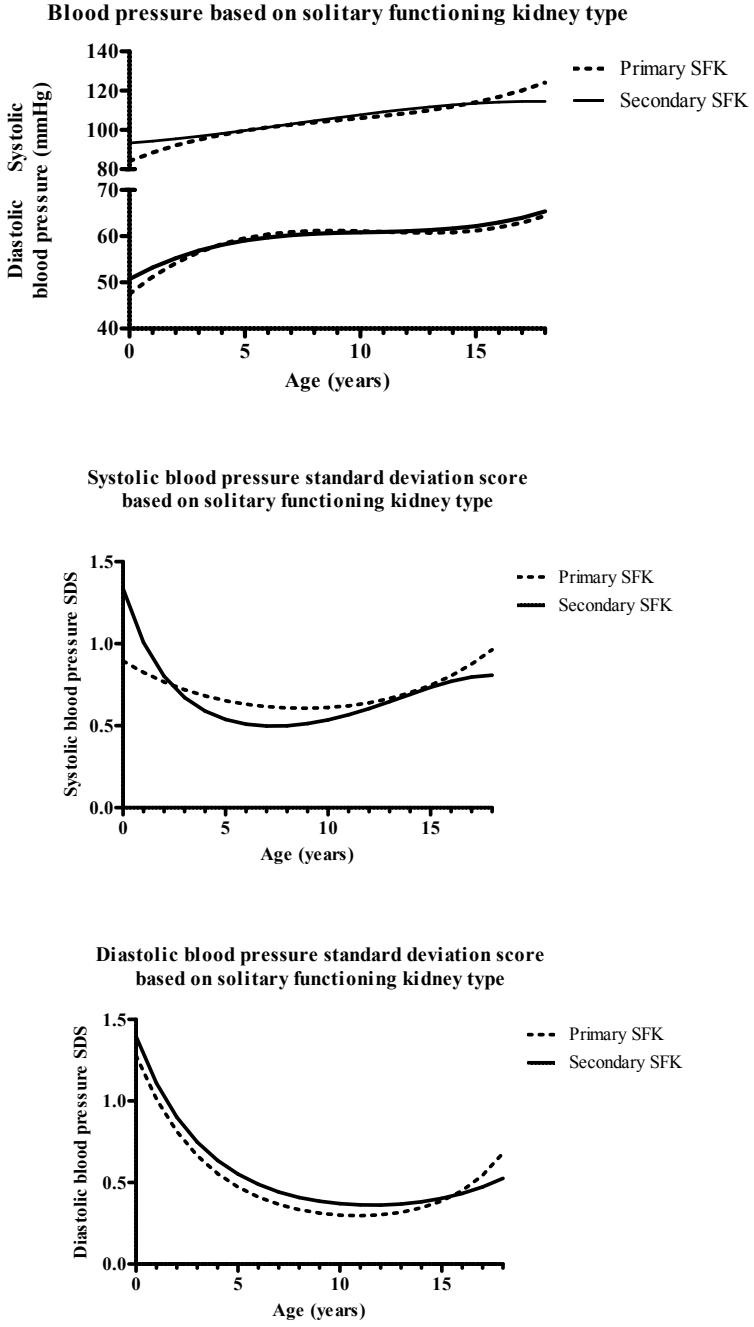
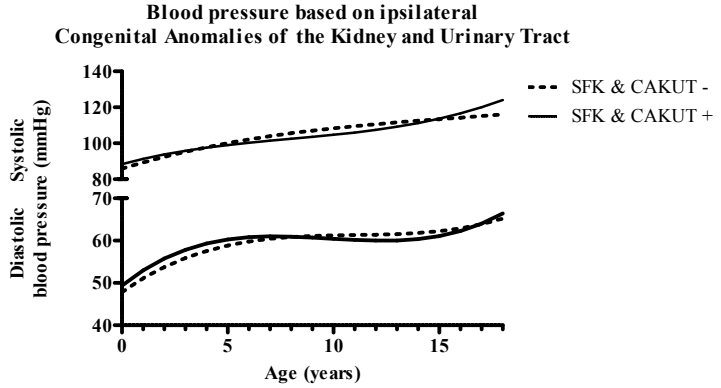
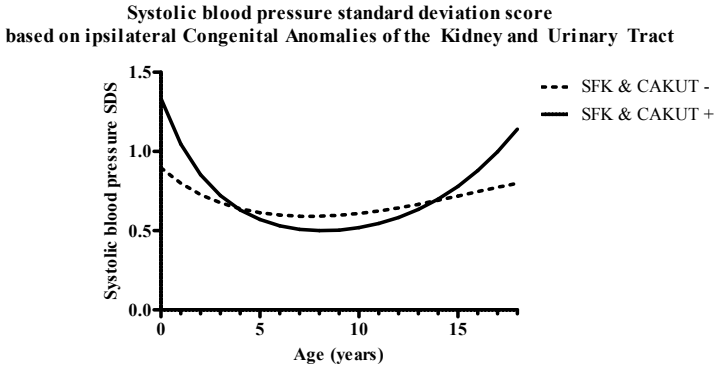


FIGURE 5.2. Generalized estimated equation-analysis on blood pressure data based on the type of solitary functioning kidney.
(**a**) Blood pressure course over childhood. (**b** and **c**) Blood pressure SDSs over childhood.





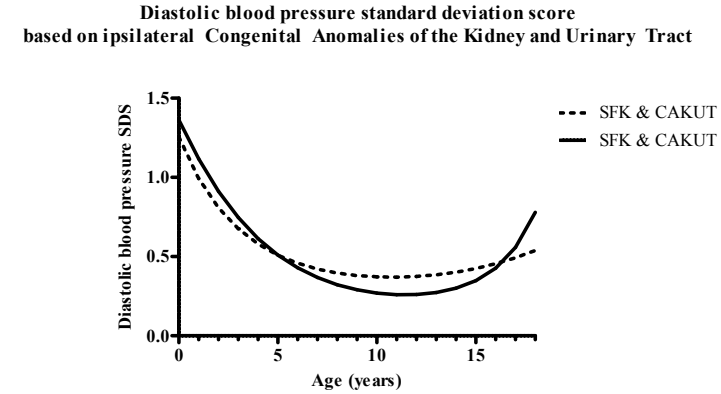


FIGURE 5.3. Generalized estimated equation-analysis on blood pressure data based on the presence of ipsilateral CAKUT.
(**a**) Blood pressure course over childhood. (**b** and **c**) Blood pressure SDSs over childhood.

GEE-analyses of blood pressure, albuminuria and eGFR

GEE-models for blood pressure based on type of SFK, are demonstrated in Figure 5.2. Progression of systolic- as well as diastolic blood pressure SDS over childhood were within normal ranges and showed an identical course between pSFK and sSFK (Figure 5.2b and 5.2c). GEE analyses for blood pressure data based on the presence of ipsilateral CAKUT did not show significant differences between groups (Figure 5.3). The clinical course of albuminuria is shown in Figure 5.4. For children with a pSFK, GEE-analysis reached microalbuminuric range at 18 years of age (22.1 μg/min). In a milder way, this

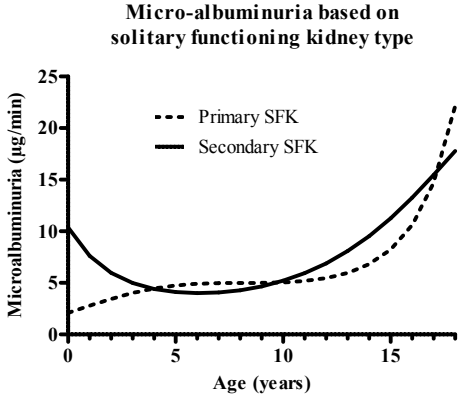


FIGURE 5.4. Generalized estimated equation-analysis on albuminuria based on the type of solitary functioning kidney.
A primary solitary functioning kidney is of congenital origin, a secondary solitary functioning kidney is acquired after unilateral nephrectomy. In this model, urinary albumin reaches micro-albuminuric range in children with a primary solitary functioning kidney at 18 years of age.

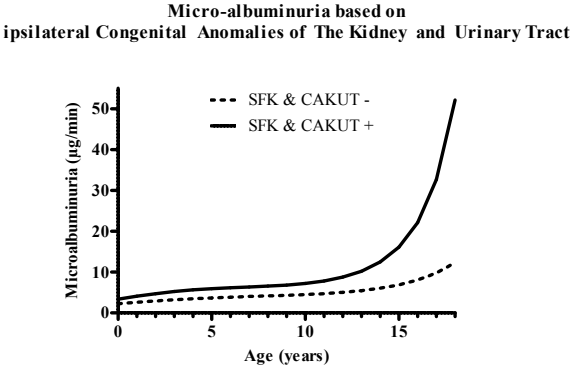


FIGURE 5.5. Generalized estimated equation-analysis on albuminuria based on the presence of ipsilateral CAKUT.
In this model, urinary albumin reaches micro-albuminuric range in children with a solitary functioning kidney and congenital anomalies of the kidney and urinary tract at 16 years of age.

course is identified in children with an sSFK (17.8 μg/min at 18 years of age). Analyses on microalbuminuria in children with ipsilateral CAKUT showed a more pronounced increase (Figure 5.5). In our model, children with an SFK and ipsilateral CAKUT develop microalbuminuria at the age of 16 years with a subsequent progressive increase, whereas children without CAKUT do not reach micro-albuminuric range within our study age range.

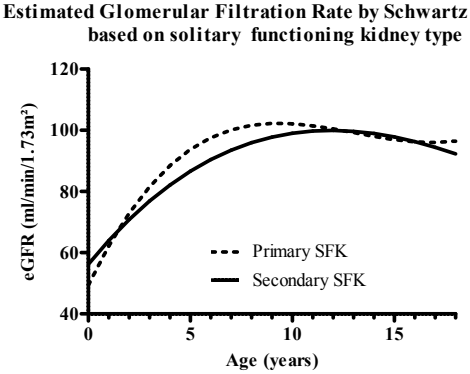


FIGURE 5.6. Generalized estimated equation-analysis on eGFR-Schwartz data based on the type of solitary functioning kidney.
A primary solitary functioning kidney is of congenital origin, a secondary solitary functioning kidney is acquired after unilateral nephrectomy. For both groups, eGFR reaches highest values at the beginning of puberty in this longitudinal model, whereas eGFR mildly declines into adolescence. eGFR by Schwartz formula.

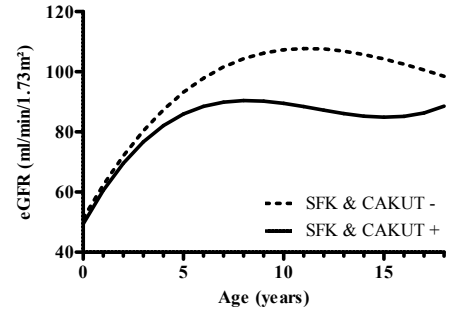


FIGURE 5.7. Generalized estimated equation-analysis on eGFR-Schwartz based on the presence of ipsilateral CAKUT.
Children with congenital anomalies of the kidney and urinary tract have a significantly lower eGFR than children without these anomalies.

Figure 5.6 shows GEE analysis of eGFR. An increase of eGFR is illustrated in both groups from infancy until the age of 9 (102 ml/min/1.73m^2) for pSFK and 11 years (96 ml/min/1.73m^2) for sSFK. From the beginning of puberty onwards, eGFR slowly decreases into late adolescence. eGFR values at 18 years of age were 96 and 92 ml/min/1.73m^2 for pSFK and sSFK, respectively (P = NS). In our analyses based on the presence of ipsilateral CAKUT, we identify the same trajectory of eGFR (Figure 5.7). However, children with ipsilateral CAKUT had a lower eGFR over childhood and reach maximum eGFR at an earlier age than children without CAKUT (P = 0.03).

DISCUSSION

Our KIMONO-study is the largest cohort of children with an SFK studied and demonstrates that nearly a third of all children with an SFK has either hypertension, albuminuria or uses renoprotective medication at a young age. Furthermore, these symptoms of renal injury are not dependent on the origin of the SFK. There is a small number of patients that already has an impaired GFR at childhood. Our data show that children with an SFK and ipsilateral CAKUT have more pronounced renal injury than children who have an SFK without additional renal tract malformations. In a longitudinal model based on GEE-analysis, we illustrate a mild decrease in eGFR from the beginning of puberty onwards following a slowly progressive course into adolescence. This concerns both groups of SFK. Our model suggests that this trend persists over adulthood and therefore might lead to chronic kidney disease in later life. Even though other reports have demonstrated this,[36,37,39] there are insufficient data available from our cohort to substantiate this at this time. Nevertheless, subsequent to these studies our results emphasize that having an SFK from childhood is not a harmless malformation, but necessitates clinical follow-up on a regular basis from childhood onwards. Since all children in our study with an impaired eGFR also showed at least one other symptom of renal injury, we propose screening for renal injury in every child with an SFK by measurement of blood pressure and urine analysis on albumin.

Ample reports have been published on glomerular hyperfiltration in children with an SFK,[38,40-42,70,130,193] all with differences regarding the origin of SFK studied, inclusion criteria and methods measuring blood pressure, albuminuria and GFR. Such studies fuel the ongoing debate on whether or not children with an SFK are prone to hyperfiltration injury in later life. Since *in vivo* methods to determine the exact nephron number are absent, it is not yet possible to establish the direct effect of glomerular hyperfiltration in patients with an SFK. Autopsy studies have demonstrated that nephron numbers are highly variable.[250] This variability implicates that the susceptibility for hyperfiltration injury ranges per individual. Another factor that hampers the study of hyperfiltration

injury in SFK is the small number of longitudinal prospective studies in children with an SFK.

In line with the study of hyperfiltration in humans, many reports have been published on donors undergoing unilateral nephrectomy as these patients are healthy subjects with normal renal function before renal mass reduction. In a large cohort of 3,698 donors who had undergone nephrectomy between 1963 and 2007, the rate of development of end-stage renal disease was lower than in the general population.[46] This is presumably the effect of the stringent screening for renal transplant donors, where all candidates with risk factors for renal disease are excluded from donation. Moreover, it is suggested that there are fundamental differences in long-term outcomes between children with an SFK and healthy adults undergoing unilateral nephrectomy regarding nephron endowment, genetics and the influence of the intrauterine environment.[49] For the study of hyperfiltration injury, we therefore hold the opinion that there is a notable difference between adult donors and children with an SFK, a statement that is supported by animal data.[251]

The high proportions of renal injury identified in our KIMONO-study underline data from a recent report on clinical outcomes in patients with CAKUT. Sanna-Cherchi et al.[2] studied 312 patients from childhood onwards, including 71 patients with a pSFK. At 30 years of age, patients with a pSFK had a nearly 50% probability to being in need of renal replacement therapy. Our GEE models for eGFR may provide an explanation for this, as eGFR deteriorates from the beginning of puberty onwards in all children with an SFK. We hypothesize that this mild decrease in renal function in the second decade is an illustration of renal maladaptation, starting when glomerular hyperfiltration is unable to be further increased at the start of puberty. Although our model demonstrates a mild and slowly progressive decline in eGFR, the vicious cycle of hyperfiltration hypothetically may come to result in a rapid decrease over time and lead to end-stage renal disease. This hypothesis is supported by a recent report by Wasilewska et al.[41] in which a subclinical impairment of GFR was identified in 44% of children over 12 years of age with a pSFK without CAKUT. In contrast to this study, children with renal injury did not have renal hypertrophy in our population at a mean age of 9 years. This may be explained by the differences in the population studied as our study population is younger and we did not exclude children with associated CAKUT, which could have further affected renal length.

In a retrospective review of adults with pSFK as well as sSFK, Argueso et al.[36,37] reported high numbers of hypertension, proteinuria and decreased GFR, even in the absence of structural abnormalities of the kidney. These studies have been the subject of debate ever since, mostly due to the presumed influence of selection bias in both studies.[3] Nevertheless, we demonstrate here that ipsilateral CAKUT in the SFK accounts for a higher prevalence of renal injury in children with a pSFK as well as an sSFK. Moreover,

our GEE-models for children with ipsilateral CAKUT show a more progressive course for microalbuminuria and eGFR. Consequently, ipsilateral CAKUT must be considered as an additional risk factor for renal injury in later life. We therefore feel that an active identification of ipsilateral CAKUT is of major importance in all children with an SFK. Renal ultrasonography should be the first step in this and can eventually be followed by voiding cystourethrography and/or renal scintigraphy.

Our study has several limitations. First, even though our data were collected using a standard protocol for testing, our cohort was evaluated retrospectively which inevitably implicates lost data. In order to minimize the effect of lost data on our results, we considered all missing data to be in the normal range. Furthermore, all patients are known at a tertiary medical center. As children with an SFK do not *per se* show clinical signs, those presenting might represent the spectrum of SFK with a poorer clinical course and prognosis. Even though this limits the generalizability of our results to all children with an SFK, our study demonstrates that a significant proportion of children with an SFK show signs of renal injury at a young age. Our analyses were based on combined groups for pSFK and sSFK. A MCDK shows complete involution in 5-74% of cases,[10] which may limit the differentiation with URA in the post-involutional period. Differences between groups may therefore be within the same spectrum of CAKUT. However, the identified differences in symptoms of renal injury in children with URA compared to children with MCDK are mostly explained by the confounding effect of a younger age in children with MCDK. Therefore, we feel that congregation of data is justified. Obstructive nephropathy and reflux nephropathy are two distinct causes of an sSFK, which must be taken into account in the interpretation of our results. A comprehensive way to exclude all selection from studying children with an SFK would be to only include children with an SFK who were diagnosed prenatally, with a normal renal function at birth. Standard prenatal ultrasonography for all pregnant women in The Netherlands has been implemented since 2006. Before this year, only women with a high risk of congenital anomalies were routinely screened. Stratification by a prenatal diagnosis therefore would only introduce more bias into our study. Nevertheless, large prospective studies of children with an SFK based on a prenatal diagnosis are highly needed. Due to the retrospective design of this study, results on infancy and late adolescence are consequently based on less available data and therefore should be interpreted with care. In greater extent, this accounts for GEE-analyses of children with ipsilateral CAKUT, which consists of a smaller sample size. Nevertheless, GEE-analysis provides a statistical method that takes into account these differences in availability of data and therefore contributes to the reliability of our models.

Blood pressure measurements were performed by an oscillometric device. This method may overestimate true blood pressure, hence overestimating the proportion of patients with hypertension. In their report on 24h-ambulatory blood pressure mea-

surements in children with renal mass reduction, Dursun et al.[38] found hypertension in 26% of patients. Seeman et al.[40] reported hypertension in 7% of children with URA and a normal renal ultrasound and 57% of children with URA and an abnormal renal ultrasound. Subsequently, our results are in line with both reports. Finally, patients who used renoprotective medication for hypertension or albuminuria were not excluded from GEE-analyses. This may suggest that the childhood trajectories for blood pressure parameters and albuminuria demonstrate a relatively milder trend that must be taken into account in the implementation of these models to all children with an SFK.

In conclusion, our study represents the largest cohort of clinically identified children with different origins of SFK studied so far. There is a large proportion of children who show signs of one or more features of renal injury at a young age. Furthermore, it is highlighted that children with an SFK and ipsilateral CAKUT have an augmented risk to develop renal injury in later life. These results once more underline that long-term longitudinal follow-up studies are urgently needed to accurately identify which patients with an SFK are at risk to develop renal injury in later life. Until then, the KIMONO-study emphasizes the importance of clinical follow-up in all children with an SFK.

Acknowledgements

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Chapter 6

The reason why mother nature provided us with two kidneys: the risks of a congenital solitary functioning kidney

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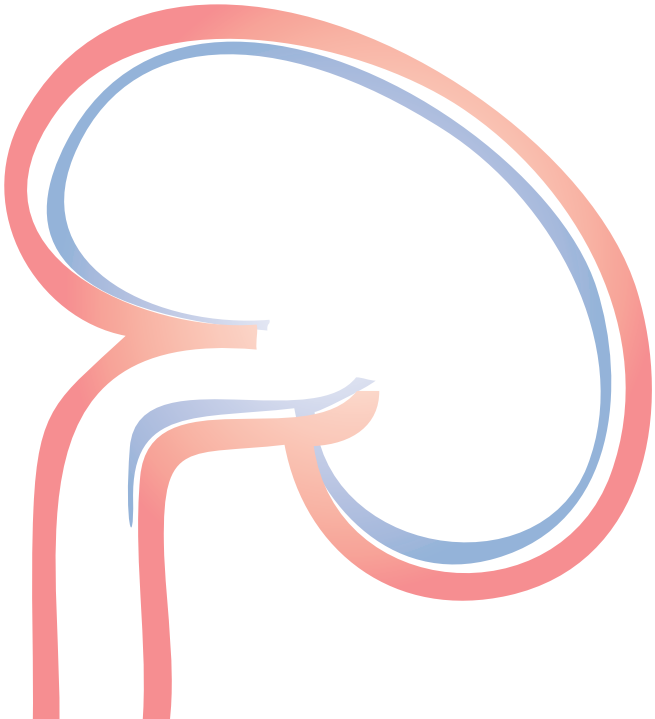

Sir,

Steiger[252] recently described a brief overview on the long-term consequences of kidney donation. Based on the landmark studies by Brenner et al.[71] glomerular hyperfiltration could be expected in individuals that donate a kidney, with long-term sequelae such as hypertension, glomerular damage with albuminuria, and chronic kidney disease. Even though glomerular hyperfiltration did take place and albuminuria did present, no difference in survival between kidney donors and control groups was present. As a conclusion, Steiger stated that '… nature gave us too much kidney mass for one lifetime…'[252]

Whereas this may hold true for the selected kidney donors, this is definitely different for individuals that are born with a reduced renal mass, such as those with a congenital solitary functioning kidney. In fact, we recently described that 32% of children with a congenital solitary functioning kidney already show signs of renal injury at a mean age of 8.4 years.[11] Follow-up has shown that ~40% of patients with a solitary functioning kidney were in renal failure at the age of 30 years,[2] a massive difference with the prospect for a kidney donor.

How can such a discrepancy be explained? The main reason may be found in the degree of glomerular hyperfiltration. Indeed, animal studies have shown that nephrectomy during nephrogenesis leads to a doubling of glomerular hyperfiltration when compared with nephrectomy at an early adult age (115% versus 47% increase in single nephron glomerular filtration rate, respectively).[47] This significant doubling of hyperfiltration is highly likely to start the vicious cycle as described by Brenner.[26] As an alternative explanation, the healthy kidney donor is evaluated in such a way that a healthy kidney will remain, whereas the disturbance in kidney development that results in a solitary functioning kidney may also lead to some degree of hypodysplasia in the remaining kidney, causing future health problems. The fact that 52% of solitary functioning kidneys did not show hypertrophy,[11] which may be expected in a healthy solitary kidney, may point in that direction. However, the incidence of renal injury in our study was similar in children with and without a hypertrophic congenital solitary functioning kidney (30% versus 33%, respectively; $P = 0.79$), so we feel that this may only play a minor role.[11]

Whatever the cause, being born with a solitary functioning kidney induces a very real health risk starting already during childhood, quite different from being born with two kidneys. Being born with two kidneys is therefore highly desirable, which may be the reason why mother nature did provide us with two.

Chapter 7

Risk factors for renal injury in children with a solitary functioning kidney

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* RW and RAJK contributed equally to this manuscript.

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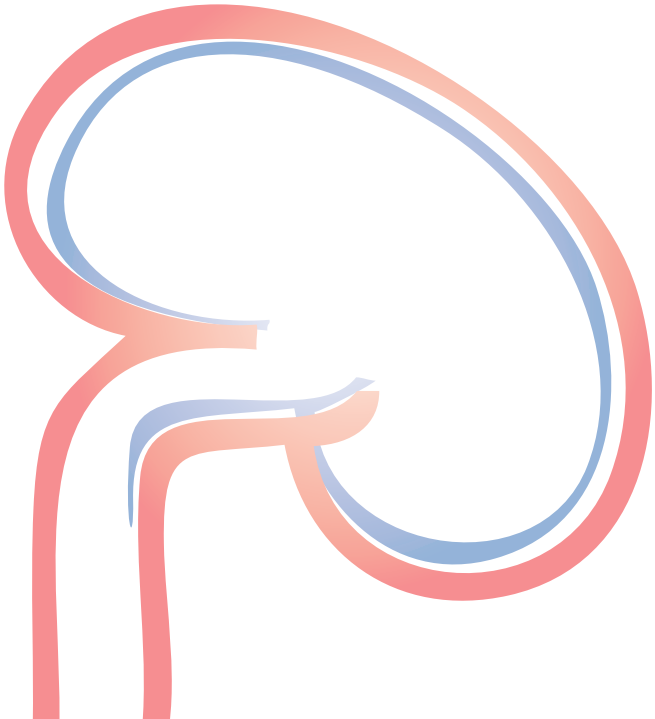

ABSTRACT

Background: The hyperfiltration hypothesis implies that children with a solitary functioning kidney are at risk to develop hypertension, proteinuria and chronic kidney disease. We sought to determine the presenting age of renal injury and identify risk factors for children with a solitary functioning kidney.

Methods: We evaluated 407 patients for signs of renal injury, defined as hypertension, proteinuria, an impaired glomerular filtration rate, and/or the use of renoprotective medication. Patients were subdivided in solitary functioning kidney-type and the presence of ipsilateral congenital anomalies of the kidney and urinary tract (CAKUT). The development of renal injury was analyzed with Kaplan-Meier-analysis. Risk factors were identified by using logistic regression models.

Results: Renal injury was found in 37% of all children. Development of renal injury increased by presence of ipsilateral CAKUT (odds ratio [OR] 1.66; $P = 0.04$) and age (OR 1.09; $P < 0.001$). Renal length was inversely associated with the risk to develop renal injury (OR 0.91; $P = 0.04$). In all patients, the median time to renal injury was 14.8 years (95% confidence interval 13.7-16.0 years). This was significantly shortened for patients with ipsilateral CAKUT (12.8 years, 95% confidence interval 10.6-15.1 years).

Conclusions: Our study determines independent risk factors for renal injury in children with a solitary functioning kidney. Because many children develop renal injury, we emphasize the need for clinical follow-up in these patients starting at birth.

INTRODUCTION

More than three decades ago, Brenner and coworkers described their hyperfiltration theory following experiments in rats with subtotal renal mass reduction.[25] Reducing the functional nephron number changed glomerular hemodynamics in remnant nephrons, and resulted in a vicious cycle of glomerular hyperfiltration and glomerulosclerosis. Renal injury due to this hyperfiltration presents as hypertension or proteinuria during the early stages but may eventually end in chronic kidney disease.[26,253]

Approximately 900,000 nephrons per kidney are formed in humans[6] with a wide interindividual variation.[254] Nephrogenesis terminates before birth, without the possibility of postnatal nephron formation.[250] Low nephron numbers have been described in patients with hypertension,[31] providing evidence for the hyperfiltration hypothesis in humans. However, methods that could confirm the hyperfiltration hypothesis in humans through *in vivo* measurement of nephron number are not yet available.

Children with a solitary functioning kidney (SFK) have renal mass reduction during an extended period, and this may allow study of the consequences of glomerular hyperfiltration in humans. Indeed, we previously showed that 32% of children with an SFK developed renal injury around 10 years of age.[11] Furthermore, a recent study demonstrated that 20-50% of the study young adults with a congenital SFK required dialysis by the age of 30 years,[2] leading to the advice to monitor all patients with an SFK from childhood.[45,49] To guide the timing and frequency of clinical follow-up, information is needed on the age at which renal injury presents and on clinical factors that differentiate between children with and without renal injury.

The KIMONO (KIdney of MONofunctional Origin)-study aims to determine the age of presentation of renal injury in children with an SFK. In addition, we identify clinical risk factors for the development of renal injury.

METHODS

Patients

All children with an SFK and renal follow-up at 2 pediatric renal centers (VU University Medical Center, Amsterdam, and Radboud University Nijmegen Medical Centre, Nijmegen) in The Netherlands were included in this retrospective longitudinal cohort study (enrollment period: 1992-2011). Part of this cohort has been previously described.[11] An SFK was identified by the unilateral absence of (functional) renal tissue on ultrasound or on renal scintigraphy.

Children with an acquired SFK as a result of renal malignancy (n=17) were excluded because of potential confounding effects from the use of nephrotoxic chemotherapy.

Also children with an estimated glomerular filtration rate (eGFR) of <30 ml/min/1.73m^2 from birth (n=11) and children who died before reaching the age of 1 year (n=17) were excluded. All remaining 407 patients with an SFK were included in the study.

To identify differences between the 2 causes of an SFK, patients were divided into categories: congenital SFK or acquired SFK. A congenital SFK can be due to unilateral renal agenesis/aplasia or to a multicystic dysplastic kidney. An SFK is acquired when children undergo nephrectomy secondary to congenital anomalies of the kidney and urinary tract (CAKUT) such as pelviureteric junction obstruction, posterior urethral valves, or vesicoureteral reflux, as well as to acute pyelonephritis or renovascular disease.

Because patients with SFKs often have additional CAKUT,[68] which would imply an additional risk of chronic kidney disease,[2,11] a subdivision was made in patients with or without ipsilateral CAKUT (i.e. on the side of the SFK). CAKUT were identified by renal ultrasound in all patients and, on indication, by voiding cystourethrogram (n=303, 74%) and/or renal scintigraphy (n=330, 81%).

Measurements

Birth weight was obtained by chart-review and divided into 5 different groups: (i) <2,500 g (n=56, 15%); (ii) ≥2,500 – <3,000 g (n=63, 17%); (iii) ≥3,000 – <3,500 g (n=111, 30%); (iv) ≥3,500 – <4,000 g (n= 87, 23%) and (v) >4,000 g (n=56, 15%). BMI (kg/m^2) was calculated from weight and height. In addition, SD scores (SDSs) were calculated based on gender, age and ethnicity according to the Fifth Dutch Growth Study.[255]

Blood pressure was measured with automated oscillometric devices with an appropriate cuff size. To minimize the effect of stress, the lowest reading of blood pressure was used. Hypertension was defined as a persistent presence of a systolic blood pressure and/or diastolic blood pressure ≥95th percentile corrected for age, gender and height.[243]

Proteinuria was defined as a protein/creatinine ratio >0.2 mg/mg (>22.6 mg/mmol) in children >2 years of age, and as >0.5 mg/mg (>56.6 mg/mmol) for children <2 years of age. Furthermore, microalbuminuria was defined as a urinary albumin >30 mg/24h to 300 mg/24 h in timed collected urine samples or as a urinary albumin/creatinine ratio of >30 mg/g to 300 mg/g in a spot (morning) sample.[256] We aggregated data on the presence of proteinuria and microalbuminuria by using the term "proteinuria".

Creatinine (µmol/l) was measured enzymatically and an eGFR was calculated by using the Schwartz equation (eGFR= *k**height/serum creatinine in [µmol/l]), with a *k*-value of 36.5.[257] An impaired eGFR was defined as an eGFR <60 ml/min/1.73m^2.

Because medication (*i.e.* angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, beta-adrenoreceptor antagonists and calcium antagonists) could be a potential confounder in the determination of hypertension as well as proteinuria and eGFR, the use of this 'renoprotective' medication was separately obtained. Even though renoprotective medication does not influence the progression of renal disease in

children with CAKUT,[258] the use of renoprotective medications was used as a surrogate marker of clinically relevant hypertension and/or proteinuria.

Renal injury was defined as hypertension and/or proteinuria and/or an impaired eGFR and/or the use of renoprotective medication.

Renal length was measured by renal ultrasound and expressed as an SDS.[246,247] Finally, relapsing urinary tract infections, *i.e.* 2 or more during follow-up, were noted in all patients.

Statistical methods

Statistical analyses were performed by using SPSS 19.0 (Chicago, IL, USA). Values are expressed as mean and standard deviation (SD) for continuous variables and percentages for qualitative variables. Differences were analyzed with the independent-samples t-test for continuous variables. In case of non-normality, a log transformation using the natural logarithmic was performed prior to analysis. Qualitative variables were compared by using the chi-square test. Data on the variables of renal injury (blood pressure, urinary protein excretion and eGFR) were considered to be within normal range when missing.

The development of renal injury was determined by using survival analysis, according to the Kaplan-Meier method. For every patient the starting point was considered to be the day of birth. The end point was defined as the date at which a patient first showed signs of renal injury. Subsequent to this end point, the analysis was right censored. Log-rank tests were used for comparison between different groups.

Logistic regression models were used to determine risk factors for the development of renal injury. Univariate analysis to explore associations with renal injury was performed for the following variables: type of SFK, ipsilateral CAKUT, side of SFK (left/right), prenatal diagnosis, birth weight, BMI SDS (linear), relapsing urinary tract infections, and renal length SDS (linear). Because birth weight was a categorical variable, we considered the category with the lowest incidence of renal injury as the reference group. We included all variables with a P-value of ≤ 0.10 for further analysis. Also gender and age (linear) were simultaneously added. In addition, multivariate associations were explored using a backward (Wald) elimination strategy (P-value < 0.10 for inclusion and P-value > 0.157 for removal [259]) in the final model. Finally, differences were considered to be statistically significant at a $P < 0.05$ in all analyses.

RESULTS

Patient characteristics

The KIMONO-study cohort consisted of 407 patients, 223 with a congenital SFK (55%) and 184 with an acquired SFK (45%) (Table 7.1). The SFK was identified by prenatal ul-

TABLE 7.1. Cause of the solitary functioning kidney in the KIMONO-Study Cohort.

Type of solitary functioning kidney	No. of Patients (%)
Congenital solitary functioning kidney	*223 (55)*
Unilateral renal agenesis/aplasia	99 (24)
Multicystic dysplastic kidney	124 (30)
Acquired solitary functioning kidney	*184 (45)*
Vesicoureteral reflux ± urinary tract infection	78 (19)
Pelviureteric junction obstruction	24 (6)
Posterior urethral valves	14 (3)
Other obstructive nephropathy	14 (3)
Duplex system	5 (1)
Ureterovesical junction obstruction	3 (1)
Renal vein thrombosis	4 (1)
Renal vein stenosis	3 (1)
Miscellaneous	39 (10)
Total	**407 (100)**

TABLE 7.2. Clinical characteristics of the KIMONO-Study Cohort.

	SFK (N=407)	Congenital-SFK group (n=233)	Acquired-SFK group (n=184)	*P*-value
Male sex, %	265 (65)	147 (66)	118 (64)	0.71
Left-sided SFK, %	202 (50)	114 (51)	88 (48)	0.51
Age at last follow-up, y	9.0 (6.0)	7.8 (5.6)	10.5 (6.0)	0.001
Ipsilateral CAKUT, %	137 (34)	59 (26)	78 (42)	0.001
Vesicoureteral reflux, %	61 (15)	24 (11)	37 (20)	0.01
>1 CAKUT, %	30 (7)	11 (5)	19 (10)	0.04
Urinary tract infection, %	133 (33)	45 (20)	88 (48)	< 0.001
Systolic blood pressure, SDS	0.5 (1.1)	0.5 (1.0)	0.6 (1.1)	0.33
Diastolic blood pressure, SDS	0.4 (1.0)	0.4 (0.9)	0.3 (1.0)	0.71
eGFR (ml/min/1.73m^2)	101 (30)	104 (28)	98 (32)	0.06
Renal length, SDS	2.8 (2.6)	3.0 (2.6)	2.5 (2.7)	0.43
Birth weight, kg	3.2 (0.8)	3.2 (0.8)	3.3 (0.7)	0.23
Body mass index, SDS	0.2 (1.3)	0.1 (1.4)	0.5 (1.2)	0.01

Data are presented as No. of patients (%) or mean (SD). For continuous variables, clinical parameters from last follow-up are shown. *P*-values represent differences between congenital-SFK and acquired-SFK. CAKUT, congenital anomalies of the kidney and urinary tract; eGFR, estimated glomerular filtration rate; SDS, standard deviation score and SFK, solitary functioning kidney.

trasound in 176 (43%) subjects. For the remaining children, the SFK was identified after renal ultrasound due to the development of symptoms (e.g. urinary tract infection, signs of renal injury, abdominal pain or a palpable abdominal mass) or by chance during an abdominal ultrasound for non-urinary tract indications.

The mean age at last follow-up was 9.0 years (SD 6.0 years) (Table 7.2). Children with an acquired SFK were older than children with a congenital SFK ($P = 0.001$). Ipsilateral CAKUT were present in 137 (34%) patients and more frequently found in the acquired SFK-group than in the congenital SFK-group ($P = 0.001$). Relapsing urinary tract infections occurred in 33% of patients (Table 7.2), with a higher incidence in the acquired SFK-group than in the congenital SFK-group ($P < 0.001$). The overall mean renal length SDS (n=389) was 2.8 (SD 2.6).

Renal injury

One hundred fifty-one (37%) patients met the criteria for renal injury, defined as hypertension and/or proteinuria and/or an impaired glomerular filtration rate and/or the use of renoprotective medication, at a mean age of 6.4 years (SD 5.9 years). At follow-up, renal injury was more frequently identified in the acquired SFK-group than in the congenital SFK-group ($P = 0.002$; Table 7.3). Children with ipsilateral CAKUT demonstrated a higher proportion of renal injury than children without ipsilateral CAKUT (49% versus 31%, respectively; $P < 0.001$). After dividing patients into quartiles according to renal length, children with the smallest SFK (SDS < 1.1) had a higher incidence of renal injury than children with the largest SFK (SDS > 4.2) (50% versus 34%, respectively; $P = 0.032$).

Hypertension was found in 107 (26%) patients, with a higher proportion in the acquired SFK-group ($P = 0.039$; Table 7.3). Patients developed hypertension at a mean age of 4.9 years (SD 5.4 years). No differences in systolic or diastolic blood pressure SDS were found between the acquired-SFK and congenital-SFK groups. Patients with hypertension were more likely to have proteinuria and an impaired eGFR and more often used

TABLE 7.3. Renal injury according to the type of SFK.

	SFK (N=407)	Congenital-SFK group (n=223)	Acquired-SFK group (n=184)	*P*-value
Renal injury	151 (37)	68 (31)	83 (45)	0.002
Hypertension	107 (26)	49 (22)	58 (32)	0.04
Proteinuria	79 (19)	29 (13)	50 (27)	< 0.001
eGFR <60 ml/min/1.73m^2	25 (6)	9 (4)	16 (9)	0.05
Renoprotective medication	80 (20)	37 (17)	43 (23)	0.09

Data are presented as No. of patients (%). *P*-values represent differences between congenital SFK and acquired SFK. eGFR, estimated glomerular filtration rate; SDS, standard deviation score and SFK, solitary functioning kidney.

renoprotective medication than did patients without hypertension (35% versus 14%, 16% versus 3% and 62% versus 5%, respectively; *P*-values < 0.001).

Ninety-seven (19%) patients were diagnosed with proteinuria at a mean age of 9.8 years (SD 5.6 years). Proportions of proteinuria were equal between patients with the 2 types of SFK. Patients with proteinuria more often used renoprotective medication (53% versus 12%, respectively; $P < 0.001$) and had a higher incidence of an impaired eGFR (20% versus 2%, respectively; $P < 0.001$) compared with patients without proteinuria.

Twenty-five (6%) children developed an impaired eGFR during follow-up (mean age 6.4 (SD 5.7) years). Mean eGFR at the last follow-up was 103 ml/min/1.73m^2 (SD 30 ml/min/1.73m^2). The acquired-SFK group showed trends for a lower eGFR at follow-up ($P = 0.056$) and a higher proportion of an impaired eGFR ($P = 0.051$) compared with the congenital-SFK group (Tables 7.2 and 7.3). Six (24%) patients with an impaired eGFR did not show other signs of renal injury. In the entire cohort, 3 (1%) children developed end-stage renal disease at 2, 6 and 15 years of age.

Renoprotective medication was used by 80 (20%) patients. Mean age at the start of treatment was 9.8 years (SD 5.5 years). In all patients, the indication to start treatment was hypertension and/or persistent presence of proteinuria during clinical follow-up.

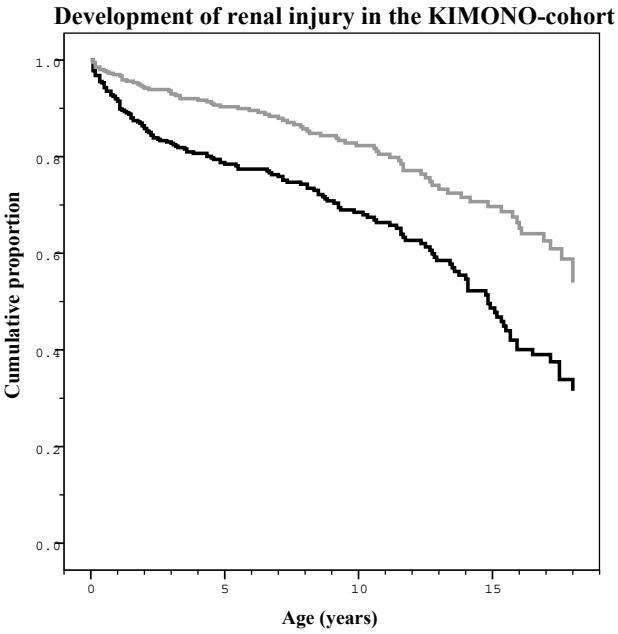

FIGURE 7.1. Kaplan-Meier curves showing the cumulative proportion to remain free from renal injury (black line) or to remain free from renoprotective medication (gray line) for children with an SFK. Renoprotective medication is depicted as a surrogate marker of clinically relevant renal injury.

Kaplan-Meier analyses

To determine the development of renal injury in children with SFK, we performed Kaplan-Meier analyses (Figures 7.1, 7.2 and 7.3). Overall median time to develop renal injury was 14.8 years (95% confidence interval [CI] 13.7 – 16.0 years). The cumulative proportions to remain free from renal injury at the end of the following intervals were: 1 year 86%, 5 years 77%, 10 years: 66% and 15 years 40%. The cumulative time to remain free from renoprotective medication (i.e. a surrogate marker for clinically relevant renal injury) is also presented in Figure 7.1.

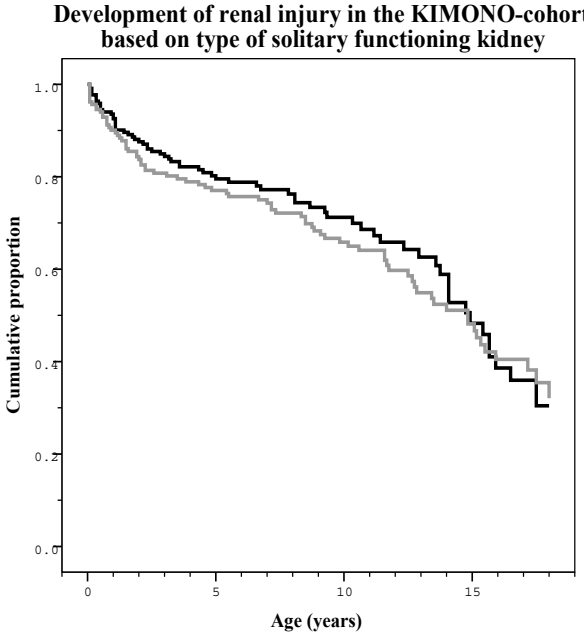

FIGURE 7.2. Kaplan-Meier curves showing the cumulative proportion to remain free from renal injury for children with a congenital SFK (black line) or with an acquired SFK (gray line) (acquired versus congenital: $P = 0.503$).

Separate analysis was performed for the congenital-SFK group (median time to develop renal injury 14.9 years, 95% CI 13.5 - 16.3 years) and the acquired-SFK group (median time to develop renal injury: 14.8, 95% CI 12.8 – 16.7 years) (Figure 7.2). The median time to develop renal injury was similar between the types of SFK ($P = 0.50$), whereas patients with CAKUT had a shorter median time to develop renal injury than did patients without CAKUT (12.8 years, 95% CI 10.6 - 15.1 versus 15.9 years, 95% CI 13.9 - 17.9 years, respectively; $P = 0.006$) (Figure 7.3).

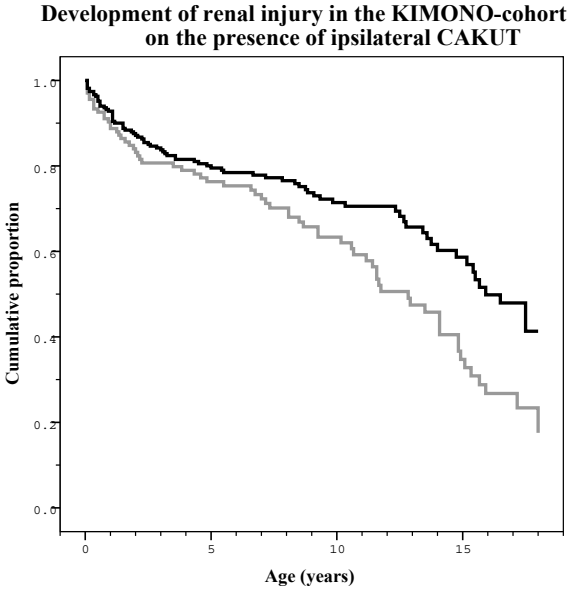

FIGURE 7.3. Kaplan-Meier curves showing the cumulative proportion to remain free from renal injury for children with an SFK (gray line) or without (black line) ipsilateral CAKUT (with CAKUT versus without CAKUT: $P = 0.006$).

TABLE 7.4. Univariate analysis of risk factors for renal injury in children with a solitary functioning kidney.

	SFK (N=357)	
	OR (95% CI)	*P*-value
Female sex	0.89 (0.57-1.39)	0.89
Age, y	1.10 (1.06-1.14)	< 0.001
Acquired SFK	1.93 (1.26-2.95)	0.002
Ipsilateral CAKUT	1.93 (1.25-2.99)	0.003
Left-sided SFK	0.95 (0.62-1.44)	0.80
Prenatal diagnosis	0.44 (0.29-0.69)	< 0.001
Birth weight <2,500 g	2.35 (1.17-4.70)	0.02
BMI SDS	1.05 (0.90-1.23)	0.51
Urinary tract infections	2.04 (1.31-3.20)	0.002
Renal length SDS	0.90 (0.82-0.98)	0.01

Variables with a *P*-value < 0.10 were included for multivariate analysis. For birth weight, as a categorical variable, the category with the lowest incidence of renal injury (≥3,500 g – <4,000 g) is used as the reference group. BMI, body mass index; CAKUT, congenital anomalies of the kidney and urinary tract; CI, confidence interval; OR, odds ratio; SDS, standard devation score and SFK, solitary functioning kidney.

Risk factor analysis

Analysis was performed on 357 children who had complete data on all potential risk factors. Univariate analysis (Table 7.4) showed an association with the development of renal injury and increasing age, acquired SFK, ipsilateral CAKUT, prenatal diagnosis, birth weight <2,500 g, history of urinary tract infections and renal length SDS. There was no association between renal injury and side of the SFK or BMI SDS. Results from

TABLE 7.5. Multivariate analysis of risk factors for renal injury in children with a solitary functioning kidney.

	SFK (N=357)	
	OR (95% CI)	*P*-value
Female sex	0.73 (0.44-1.22)	0.23
Age (years)	1.09 (1.04-1.13)	< 0.001
Ipsilateral CAKUT	1.66 (1.02-2.69)	0.04
Birth weight <2,500g	2.08 (0.96-4.51)	0.07
Urinary tract infections	1.56 (0.94-2.58)	0.08
Renal length SDS	0.91 (0.83-1.00)	0.04

Variables with a *P*-value < 0.05 were considered as independent risk factors in the development of renal injury. For birth weight, as a categorical variable, the category with the lowest incidence of renal injury (≥3,500 g – <4,000 g) is used as the reference group. CAKUT, congenital anomalies of the kidney and urinary tracts; CI, confidence interval, OR, odds ratio; SDS, standard devation score and SFK, solitary functioning kidney.

the multivariate analysis are shown in Table 7.5. After adjustments, increasing age and the presence of ipsilateral CAKUT were shown to be independent risk factors for renal injury (Table 7.5). In addition, renal length SDS was inversely associated with the risk to develop renal injury. Birth weight <2,500 g and a history of urinary tract infections were associated with renal injury, although differences were not statistically significant (P = 0.065 and P = 0.083, respectively). Type of SFK (odds ratio [OR] 1.39, 95% CI 0.85 – 2.28; P = 0.19) and prenatal diagnosis (OR 0.77, 95% CI 0.82 – 2.25; P = 0.77) did not show to be independent risk factors for the development renal injury.

DISCUSSION

The KIMONO-study demonstrates that a substantial proportion of children with an SFK develop renal injury during childhood. Kaplan-Meier-analysis showed that these children have a median time toward renal injury of ~15 years. Renal injury development is independent of SFK-type, but is significantly accelerated when there is additional ipsilateral CAKUT. In addition, insufficient renal hypertrophy can be considered as an

independent risk factor for renal injury. Low birth weight and urinary tract infections demonstrate a trend in the association with renal injury.

We previously described a similar incidence of renal injury in a smaller cohort of patients with SFK.[11] Although similar in design, our cohort has doubled compared to the first KIMONO-Study, allowing for regression analyses and more robust conclusions. Also, with the use of Kaplan-Meier analysis, this study demonstrates the presenting age of renal injury in these specific patients.

As reported in patients with diabetes,[260] we expected renal injury to occur in the second decade after the onset of glomerular hyperfiltration. Surprisingly, our results illustrate that patients show signs of renal injury during the full age range of childhood. Twenty-three percent of patients already were symptomatic before the age of 5 years. We hypothesize that these are children with a certain degree of (hypo)dysplasia in the SFK, which results in signs at an earlier stage due to a subsequent insufficient nephron endowment or inappropriate renin-angiotensin system activation. Follow-up of children with an SFK should therefore start at a young age. Subsequently, many patients with an SFK developed signs in the second decade. This might be a reflection of glomerular hyperfiltration necessary to maintain normal eGFR during ongoing increases in body surface area and associated metabolic demands.

We found a higher incidence of renal injury in the acquired-SFK group than in the congenital-SFK group. Nevertheless, our multivariate analysis and Kaplan-Meier analysis demonstrated that the development of renal injury is independent of SFK-type. We therefore suggest that the impaired renal outcome in acquired SFK is caused by the older age and the higher incidence of CAKUT, which are risk factors for renal injury. All in all, we now unequivocally show that an SFK, either congenital or acquired, should not be considered to be a harmless malformation.

Although the effect was small, we show that insufficient compensatory renal hypertrophy implies a higher risk to develop injury. This might be caused by inadequate nephrons numbers and/or dysplastic components in the smaller SFK. Birth weight is another potential risk factor that should be considered during clinical follow-up. Although our model could not fully substantiate low birth weight as an independent risk factor for renal injury, other studies have associated lower birth weight with a decreased nephron number.[261,262] Such studies could provide evidence for a combination of the developmental origins of health and disease hypothesis[263,264] and the hyperfiltration hypothesis in children with an SFK.[5,265]

Studies on long-term outcomes of children with an SFK have conflicting resul ts.[2,3,11,35-42,65,70,130,193,242] In addition, it is often stated that children with an SFK are not expected to develop renal damage, because most long-term follow-up surveys on kidney donors show an excellent prognosis.[46] However, hyperfiltration is much more pronounced when renal mass reduction occurs earlier in life,[47] which makes these situ-

ations (SFK in childhood versus uninephric kidney donors) not comparable. Moreover, the excellent prognosis of kidney donors could result from the stringent screening before donation.[46,266]

Our results are complementary to survival analyses by Sanna-Cherchi et al.[2] on renal outcome in adults with different types of CAKUT. They demonstrated that adults with a congenital SFK show a high incidence of end-stage renal disease at 30 years of age. As a consequence, their findings emphasize the lifelong need for regular follow-up of individuals with an SFK.[49] Corbani et al.[45] propose annual laboratory tests for kidney function, urinary analysis and blood pressure measurement in children with ipsilateral CAKUT and the same regime every 2 years for children without ipsilateral CAKUT. If the latter remain asymptomatic until puberty, the follow-up is expanded to once every 3 to 5 years. However, our results may implicate at least annual follow-up of children with SFK until adulthood.

Our results should be interpreted with respect to the retrospective study design. First, to minimize potential overestimation of renal injury, we considered missing data to be in the normal range. Second, our cohort is followed at 2 tertiary medical centers, which might implicate selection bias. However, multivariate analysis on renal injury for all children with a prenatal diagnosis, as the best surrogate marker of an unbiased group of SFK, did not reach statistical significance. Third, we were unable to determine the direct effect of glomerular hyperfiltration on the development of renal injury as this can only be measured in animal studies. One morphometric study on glomerular size indeed has reported an increased glomerular volume in patients with a congenital solitary kidney, indicating glomerular hyperfiltration.[59] Nevertheless, other potential causes for renal injury must be considered in the interpretation of our data. Fourth, blood pressure was measured by using oscillometric devices, which may overestimate the proportion of children with hypertension and, consequently, the proportion of renal injury. Our retrospective design also hampered us to determine the influence of white coat hypertension and other causes of hypertension in our cohort. Nevertheless, our results are in line with studies on hypertension in SFK by using ambulatory blood pressure monitoring.[38,40,130] Finally, because our markers for renal injury are indirect and may therefore be somewhat insensitive, we emphasize the need for new markers for early renal damage. Fibroblast growth factor 23 and cystatin C have been identified as promising markers in SFK.[41,267]

CONCLUSION

The KIMONO-study demonstrates that a substantial proportion of children with an SFK develop renal injury during childhood. This risk to develop renal injury is independent of SFK-type, but increases with the presence of ipsilateral CAKUT, age and a small renal

length. Identification of these risk factors is important, together with regular follow-up of blood pressure, proteinuria and eGFR. Because renal injury in SFK may develop from infancy on, clinical follow-up of every child with an SFK should start at birth.

Acknowledgments

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Chapter 8

Gender differences in solitary functioning kidney: do they affect renal outcome?

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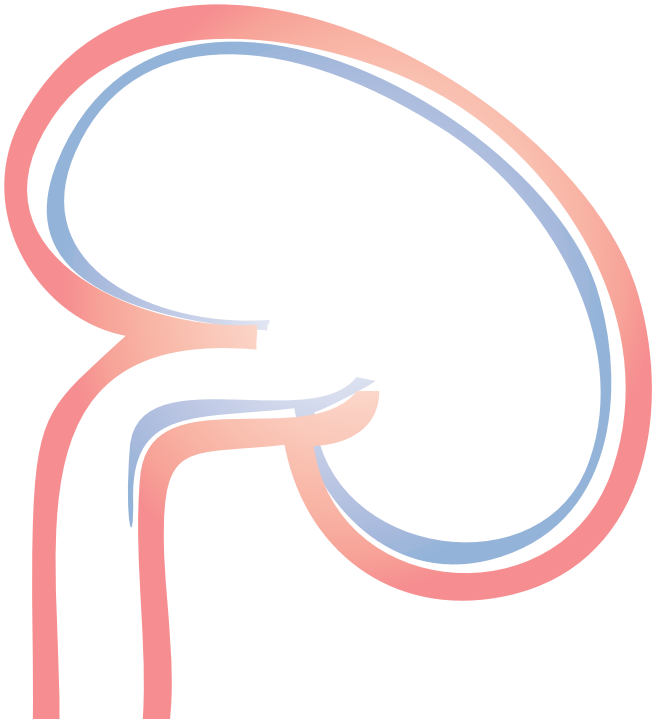

Dear Sir,

Kummer et al.[268] recently reviewed the influence of gender and sex hormones on the incidence and outcome of chronic kidney disease (CKD). The authors described the marked differences between males and females in the prevalence and timing of CKD. Renal failure in infancy and childhood is more frequently identified in boys due to the higher prevalence of congenital anomalies of the kidney and urinary tract (CAKUT), whereas females show a rapid increase in CKD at the beginning of the menopause when sex hormone levels are declining. Since genetic factors and not hormonal influences are presumed to play a major role in CAKUT, the authors chose not to review the gender differences in CAKUT-patients.

We have recently published results from our KIMONO-study on the development of renal injury in over 400 children with different types of solitary functioning kidney (i.e. a frequent CAKUT-phenotype).[13] In line with Kummer et al.,[268] our KIMONO cohort consisted mainly of boys (65% versus 35% girls). Overall, no differences were noted in the prevalence of renal injury, defined as hypertension, proteinuria, antihypertensive/antiproteinuric medication or impaired glomerular filtration rate (GFR), between both groups (boys 39% versus girls 35%; $P = 0.43$). Sub-analysis of renal injury in post-pubertal patients only (defined as ≥15 years of age) identified similar proportions (boys 65% versus girls 56%; $P = 0.43$). Multiple regression analysis could not identify female gender as a protective factor in the development of renal injury (odds ratio 0.73, 95% confidence interval (CI) 0.44 – 1.22; $P = 0.23$). Furthermore, Kaplan-Meier analysis showed no differences in the median age at presenting renal injury between boys (14.7 years, 95% CI 13.4 –15.9) and girls (15.4 years, 95% CI 12.3 – 18.6; $P = 0.49$). In contrast, 80% of the children with an impaired GFR (i.e. <60 ml/min/1.73m^2) were male, which showed a trend towards a significantly higher-than-expected proportion (0.65) males (corrected z-score = 1.36; $P = 0.09$).

On the basis of our results, no clear gender differences in the development of renal injury are present in children with a solitary functioning kidney. However, boys are more frequently affected by CAKUT and may also have a more severe phenotype leading to an earlier impairment of GFR. Although we could not yet substantiate this in our post-pubertal patients, Wühl et al. have indeed reported that males with CAKUT develop end-stage renal disease at a younger age than females.[1] Follow-up on our KIMONO cohort will show whether gender differences come to light at a progressing age.

Although the exact mechanisms within the gender differences in CKD still remain largely unknown, the review by Kummer et al.[268] is of great importance to create awareness among clinicians for differences in disease progression between patients. We hope that new studies on gender differences will contribute to an improved outcome for all CAKUT-patients, both male and female.

Acknowledgments

We would like to thank Dr. Joanna A.E. van Wijk for her outstanding mentorship and contribution to the KIMONO-study.

Chapter 9

Precision of estimating equations for GFR in children with a solitary functioning kidney

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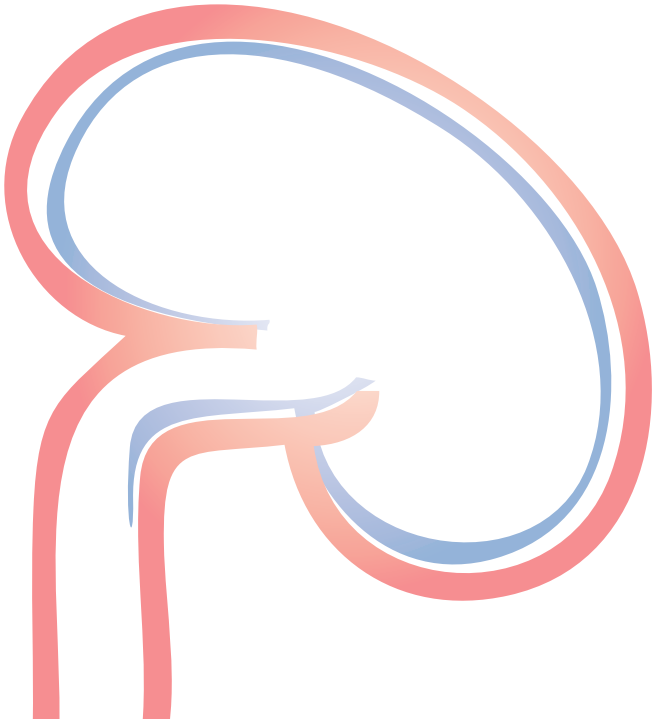

ABSTRACT

Background: Children with a solitary functioning kidney may develop chronic kidney disease. Although widely used, equations to estimate glomerular filtration rate (GFR) are not validated in these patients. This study sought to determine the precision of common estimating equations in the KIMONO (KIdney of MONofunctional Origin) cohort.

Methods: Two creatinine-based (*estimated GFR [eGFR]-Schwartz*, urinary creatinine clearance), two cystatin C-based (*eGFR-Zappitelli1, eGFR-CKiD* [Chronic Kidney Disease in Children] 1), and two cystatin C/creatinine-based (*eGFR-Zappitelli2, eGFR-CKiD2*) estimates were compared with the gold standard GFR measured by inulin single-injection method (*GFR-inulin*) in 77 children with a solitary functioning kidney (time span of assembly, 2005-2012). Included patients were between 1.5-19.8 years of age. Kidney Disease Outcomes Quality Initiative (K/DOQI) classification was compared between GFR-inulin and eGFR-methods to analyze misclassification by estimating equations.

Results: The *eGFR-CKiD2* equation performed best in children with a solitary functioning kidney (mean bias –0.9 ml/min per 1.73m^2; 95% and 54% of values within ±30% and ±10% of *GFR-inulin*, respectively). Mean bias for *eGFR-Schwartz* was 0.4 ml/min per 1.73m^2, with 90% and 33% of values within ±30% and ±10% of *GFR-inulin*, respectively. For all estimates, misclassification in K/DOQI-stage ranged from 22% (*eGFR-Zappitelli1*) to 44% (urinary creatinine clearance) of children.

Conclusions: Use of a combined serum cystatin C/creatinine-based equation (*eGFR-CKiD2*) is recommended to monitor renal function in children with a solitary functioning kidney. When cystatin C is not routinely available, *eGFR-Schwartz* should be used. Misclassification in K/DOQI-stage remains a caveat for all equations.

INTRODUCTION

According to the hyperfiltration hypothesis, a reduction in renal mass results in an ongoing loss of nephrons due to changed intraglomerular hemodynamics.[25,26,71] In the long run, glomerular hyperfiltration will lead to hypertension and (micro)albuminuria and may eventually result in a decrease in glomerular filtration rate (GFR).

Children with a solitary functioning kidney (SFK) are a clinically important example of renal mass reduction because they are likely to be exposed to glomerular hyperfiltration for an extended period of time. Although the hyperfiltration hypothesis has never been confirmed in humans, a recent study indeed demonstrated that 20-50% of adults with an SFK from childhood required dialysis by the age of 30 years.[2] These results prompted to recent recommendations to monitor all patients with an SFK from childhood,[11,45,49] which includes urine analysis and blood pressure measurements, and a determination of GFR as the best overall measurement of renal function.

Unfortunately, a gold standard measurement of GFR by inulin clearance is cumbersome, costly and therefore not commonly available.[269] In daily pediatric care, equations that use creatinine to estimate GFR (eGFR) have been widely adopted by clinicians, as recommended in the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines.[270] One such equation is the "original" Schwartz-formula (*eGFR-Schwartz*), which uses serum creatinine, height and an empirical constant (*k-value*). The *eGFR-Schwartz* was devised in the mid 1970s[271] and was recalibrated in 2009.[257] Another frequently used surrogate measure for GFR is the urinary creatinine clearance (*Ccreat*), which is based on timed urine collections.

Among others, a major disadvantage of GFR estimation using serum creatinine is the fact that this molecule is not only eliminated by glomerular filtration but also secreted in the proximal tubule. Because tubular secretion of creatinine is highly variable and increases with declining GFR,[272] the accuracy of the creatinine-based equations is limited when GFR decreases. Furthermore, serum creatinine concentrations are influenced by muscle mass,[273] which may hamper the interpretation of serum values in the growing child.[245] In addition, timed urine collections may be imprecise because of improper urine collection, in particular in children who are not yet fully toilet trained.

The shortcomings of serum creatinine have initiated the search for new endogenous markers for CKD.[274] Of these, cystatin C, a serum protein that is freely filtered by the glomerulus and not secreted or reabsorbed intact in the renal tubule, is the most promising.[275] Serum cystatin C is independent of sex, muscle mass and dietary protein intake[276] and is indeed a more precise marker for early CKD than serum creatinine.[277] This finding led to the development of eGFR formulas based on serum cystatin C in the last decade.[257,278-282] For example, Schwartz et al. have recently introduced a multivariate equation using height, gender, serum creatinine, cystatin C and blood urea nitrogen

(BUN; *eGFR-CKiD [Chronic Kidney Disease in Children] 2*) in addition to the univariate original *eGFR-Schwartz*.[282]

Nevertheless, it must be noted that all currently used estimating equations for GFR have been validated only in children with two kidneys. It is well known that the performance of estimating equations depends on the population in which the respective equation was calibrated.[283] Furthermore, glomerular hyperfiltration could hypothetically lead to an altered renal handling of endogenous markers. In line with this hypothesis, Tan et al.[284] showed that the serum creatinine-based eGFR equations lead to misclassification in stage of chronic kidney disease (CKD) in adult uninephric kidney donors (i.e. an example of SFK). Thus, it is important that common estimating equations are validated before being used in children with an SFK.

Therefore, the KIMONO (KIdney of MONofunctional Origin) study examined the precision of six common estimating equations in predicting gold standard GFR, determined by an inulin single-injection method, in children with an SFK.

METHODS

Study patients

The study protocol adhered to the principles of the Declaration of Helsinki and was approved by the ethics committee of the VU University Medical Center. Eligible participants were all patients with an SFK known at the Pediatric Renal Center of the VU University Medical Center. Patients participated in the KIMONO-study,[11] a large cohort-study in children with an SFK, or underwent GFR measurement on the basis of clinical indications (time span of cohort assembly, May 2005-May 2012). Patients with a renal transplant and those who used glucocorticosteroids were excluded because of potential interactions with cystatin C metabolism.[285]

SFK was diagnosed according to unilateral absence of functional renal tissue on ultrasonography (n=77 [100%]) and/or on renal scintigraphy (n=56 [73%]). A congenital SFK can be due to unilateral renal agenesis/aplasia or to a multicystic dysplastic kidney. An SFK can also result from renal disease that leads to unilateral nephrectomy in childhood. Patients with acquired SFK can be subdivided into three major groups: reflux nephropathy (including chronic pyelonephritis), obstructive nephropathy (including pelviureteric junction obstruction, ureterovesical junction obstruction, posterior urethral valves, ureteroceles and a duplex kidney) and postnephrectomy after renal malignancy. Renal venous thrombosis, renovascular stenosis or trauma can also result in an acquired SFK.

Measurements

For all study subjects, height (m) and weight (kg) were measured and body mass index (kg/m^2) was calculated as the weight divided by the height squared. SD scores were calculated based on the Fifth Dutch Growth Study.[255]

GFR was measured by the inulin single-injection method, which has been proven to be an accurate method to determine true GFR (*GFR-inulin*) in children.[269] All patients received a single intravenous dose (5000 mg/1.73m^2 of body surface area with a maximum dose of 5000 mg) of inulin (Inutest, Fresenius, Bad Homburg, Germany), which was administered within 1 minute. During administration, dedicated nurses assessed for extravasation of inulin, which did not occur in any of the patients included. Serial blood samples were obtained at 10, 30, 90 and 240 minutes after injection of inulin. After sampling, blood was centrifuged at 3000 rotations per minute for 10 minutes and serum was stored at –20°C until measurement. Inulin was measured within 14 days using an enzymatic method based on the determination of fructose after acid hydrolysis of inulin as described by Jung et al.[286] with some minor modifications.[287] *GFR-inulin* (ml/min per 1.73m^2) was calculated based on a two-compartmental model with MW/Pharm 3.5 software (Mediware, Groningen, The Netherlands), a pharmacokinetic program using a Bayesian estimate from patient and population data.[269]

During the clearance study, blood was drawn for measurement of serum creatinine (mg/dl), which was determined using an enzymatic method (Modular analytics <P>, Roche diagnostics, Mannheim, Germany) and traceable to isotope dilution mass spectrometry (IDMS).[288] The coefficient of variation of the creatinine assay was 2.1% (mean, 1.54 mg/dl, n=21). A calibrator for automated systems (catalog no. 10759350) was used according to the manufacturer's instructions. In 12 (16%) patients, creatinine was measured by the kinetic Jaffé method and converted to IDMS standard, as previously published.[289] Twenty-four-hours urine was collected on the day before measurement. Parents were instructed about urine collection by a dedicated nurse and received a leaflet explaining the technique.

We obtained an extra blood sample for determination of serum cystatin C levels in all children. Immediately after sampling, blood was centrifuged and serum was stored at –20°C until measurement. Cystatin C (mg/l) was measured out within one run for all samples using a particle-enhanced immunonephelometric assay (PENIA; Siemens Healthcare, Marburg, Germany) on a Behring Nephelometer II. The intra-assay coefficients of variation of the cystatin C assay were 2.3% (mean, 0.98 mg/l, n=20) and 2.9% (mean, 2.01 mg/l, n=20), respectively. We used the commercially available calibration material PROT3 CAL (no. KC770). A serum cystatin C level >0.95 mg/l was considered to be increased.

The following creatinine-based equations were used to calculate GFR[257]:

$$eGFR\text{-}Schwartz\ (\text{ml/min/1.73m}^2) = 41.3 \times \frac{height\ (m)}{serum\ creatinine\ (mg/dl)}$$

and:

$$Ccreat\ (\text{ml/min/1.73m}^2) = \frac{urine\ creatinine\ (mg/dl)}{serum\ creatinine\ (mg/dl)} \times \frac{urine\ volume\ (ml)}{time\ (hours) \times 60} \times \frac{1.73}{body\ surface\ area\ (m^2)}$$

Cystatin C-based GFR estimates were calculated according to the first Zappitelli-formula[279] and the recently improved formula derived from the CKiD-study cohort[282]:

$$eGFR\text{-}Zappitelli1\ (\text{ml/min/1.73m}^2) = \frac{75.94}{serum\ cystatin\ C\ (mg/l)^{1.17}}$$

and:

$$eGFR\text{-}CKiD\ 1\ (\text{ml/min/1.73m}^2) = 40.6 \times \left(\frac{1.8}{serum\ cystatin\ C\ (mg/l)}\right)^{0.93}$$

To evaluate equations that combine both serum cystatin C and serum creatinine to estimate GFR, we also tested the second Zappittelli-formula[279] and the *eGFR-CKiD2*[282]:

$$eGFR\text{-}Zappitelli2\ (\text{ml/min/1.73m}^2) = \frac{507.76 \times e^{0.3 \times height\ (m)}}{serum\ cystatin\ C\ (mg/l)^{0.635} \times (serum\ creatinine\ (mg/dl) \times 88.4)^{0.547}}$$

and:

$$eGFR\text{-}CKiD\ 2\ (\text{ml/min/1.73m}^2) = 39.8 \times \left(\frac{height\ ()}{serum\ creatinine\ (mg/dl)}\right)^{0.456} \times \left(\frac{1.8}{serum\ cystatin\ C\ (mg/l)}\right)^{0.418} \times \left(\frac{30}{blood\ urea\ nitrogen\ (mg/dl)}\right)^{0.079} \left[\times (1.076)^{male} \times \left(\frac{height\ (m)}{1.4}\right)^{0.179}\right]$$

On the basis the results of *GFR-inulin*, patients were classified according to the NKF-K/DOQI guidelines for CKD[270] as CKD stage 1 (GFR ≥90 ml/min per 1.73m²), stage 2 (GFR 60-89 ml/min per 1.73m²) stage 3 (GFR 30-59 ml/min per 1.73m²), stage 4 (GFR 15-29 ml/min per 1.73m²) and stage 5 (GFR <15 ml/min per 1.73m²).

The K/DOQI classification was compared with the respective classification obtained using the different estimating equations. We considered underestimation of CKD stage (i.e. CKD stage$_{GFR\text{-}inulin}$ higher than CKD stage$_{\text{estimating equation}}$) as the clinically most relevant because this implies that the estimating equation would not identify progressed CKD.

Statistical analyses

All analyses were performed using SPSS software version 18.0 (Chicago, IL, USA). Values are expressed as mean ±SD or as median (interquartile range) for continuous variables and percentages for qualitative variables. Normality of data was determined using normality plots and the Kolmogorov-Smirnov test. For continuous variables, differences between types of SFK were analyzed with the independent-samples t-test. In case of non-normality, a logarithmic transformation was performed before analysis. Qualitative variables were compared using the chi-squared test.

Using Bland-Altman analysis,[290] we calculated the bias (*GFR-inulin* minus GFR-estimating equation) for all equations and determined the 95% limits of agreement (LOAs) (i.e. mean bias ±1.96×SD). In addition, we determined the proportions of eGFR values within ±10% and within ±30% of *GFR-inulin* for all estimating equations. Underestimation in CKD stage was considered as CKD stage$_{GFR\text{-}inulin}$–CKD stage$_{\text{estimating equation}}$ ≥1. Differences were considered to be statistically significant at a *P*-value < 0.05 for all analyses.

RESULTS

Patient characteristics

Seventy-seven children with an SFK (26 [34%] with a congenital SFK and 51 [66%] with an acquired SFK) were included in this study (Table 9.1). The median age at the time of study was 14.6 (interquartile range 10.4-17.6; range 1.5-19.8) years (Table 9.2). There

TABLE 9.1. Patient distribution according to cause of the solitary functioning kidney.

Type of solitary functioning kidney	Number of patients (%)
Congenital solitary functioning kidney	*26 (34)*
Unilateral renal agenesis/aplasia	13 (17)
Multicystic dysplastic kidney	13 (17)
Acquired solitary functioning kidney	*51 (66)*
Vesicoureteral reflux ± acute pyelonephritis	28 (36)
Obstructive nephropathy	13 (17)
Renal malignancy	10 (13)
Total	**77 (100)**

Obstructive nephropathy includes pelviureteric junction obstruction (n=8), posterior urethral valves (n=3), duplex kidney (n=1) and ureterovesical junction obstruction (n=1). The group renal malignancy encompasses Wilms' tumor (n=9) and mesoblastic nephroma (n=1).

was a male predominance (n=48 [62%]). Mean height, weight and body mass index SD scores were within normal ranges for both types of SFK.

Renal function in children with an SFK

Measures of renal function are displayed in Table 9.2. In all patients, mean *GFR-inulin* was 82±24 (range, 23-126) ml/min per 1.73m^2. Supplemental Figure 9.1 presents the distribution of *GFR-inulin* by age. There was no difference in mean *GFR-inulin*, *eGFR-Zappitelli1*, *eGFR-Zappitelli2* or *eGFR-CKiD1* between SFK types. This was not the case for *Ccreat* and *eGFR-CKiD2*, which showed a lower mean eGFR for children with an

TABLE 9.2. Patient characteristics of the KIMONO-study cohort.

	SFK	Congenital SFK	Acquired SFK	*P*-value
Patients, n (%)	77 (100)	26 (34)	51 (66)	-
Male, n (%)	48 (62)	20 (77)	28 (55)	0.06
Median age (yr)	14.6 [10.4-17.6]	13.7 [10.4-17.4]	15.7 [10.3-17.5]	0.46
Height SD score	−0.7 (1.2)	−0.5 (1.2)	−0.8 (1.2)	0.25
Weight SD score	0.1 (1.6)	0.2 (1.7)	0.1 (1.6)	0.70
Body mass index SD score	0.6 (1.5)	0.5 (1.4)	0.6 (1.6)	0.34
Body surface area (m^2)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	0.94
Renal parameters				
Median serum creatinine (mg/dl)	0.81 [0.63-0.97]	0.67 [0.56-0.95]	0.85 [0.70-1.04]	0.18
Median serum cystatin C (mg/l)	0.88 [0.81-1.06]	0.89 [0.76-0.96]	0.88 [0.82-1.07]	0.81
Blood urea nitrogen (mg/dl)	14.6 [12.0-17.1]	14.0 [10.8-16.7]	14.8 [12.0-17.1]	0.45
GFR-inulin (ml/min per 1.73m^2)	82 (24)	85 (23)	81 (25)	0.43
eGFR-Schwartz (ml/min per 1.73m^2)	82 (27)	90 (27)	78 (26)	0.07
Ccreat (ml/min per 1.73m^2)	112 (46)	128 (50)	104 (42)	0.03
eGFR-Zappitelli1 (ml/min per 1.73m^2)	85 (24)	88 (26)	83 (24)	0.45
eGFR-Zappitelli2 (ml/min per 1.73m^2)	85 (26)	92 (28)	82 (24)	0.27
eGFR-CKiD1 (ml/min per 1.73m^2)	80 (20)	82 (21)	79 (20)	0.46
eGFR-CKiD2 (ml/min per 1.73m^2)	83 (22)	90 (23)	80 (21)	0.05
CKD stage 2 or higher, n (%)*	44 (57)	15 (58)	29 (57)	0.73
Increased serum cystatin C, n (%)	27 (35)	8 (31)	19 (37)	0.57

Data are presented as Number of patients (%) or as Mean (Standard Deviation) / Median [Interquartile Range]. *P*-values represent differences between congenital SFK and acquired SFK. *, Based on GFR-inulin. Ccreat, urinary creatinine clearance; CKD, chronic kidney disease; eGFR-CKiD1, estimated glomerular filtration rate based on serum cystatin C; eGFR-CKiD2, estimated glomerular filtration rate based on serum cystatin C, creatinine and blood urea nitrogen; eGFR-Schwartz, estimated glomerular filtration rate based on serum creatinine; eGFR-Zappitelli1, estimated glomerular filtration rate based on serum cystatin C; eGFR-Zappitelli2, estimated glomerular filtration rate based on serum cystatin C and creatinine; GFR-inulin, glomerular filtration rate based on inulin single-injection method; SDS, standard deviation score and SFK, solitary functioning kidney.

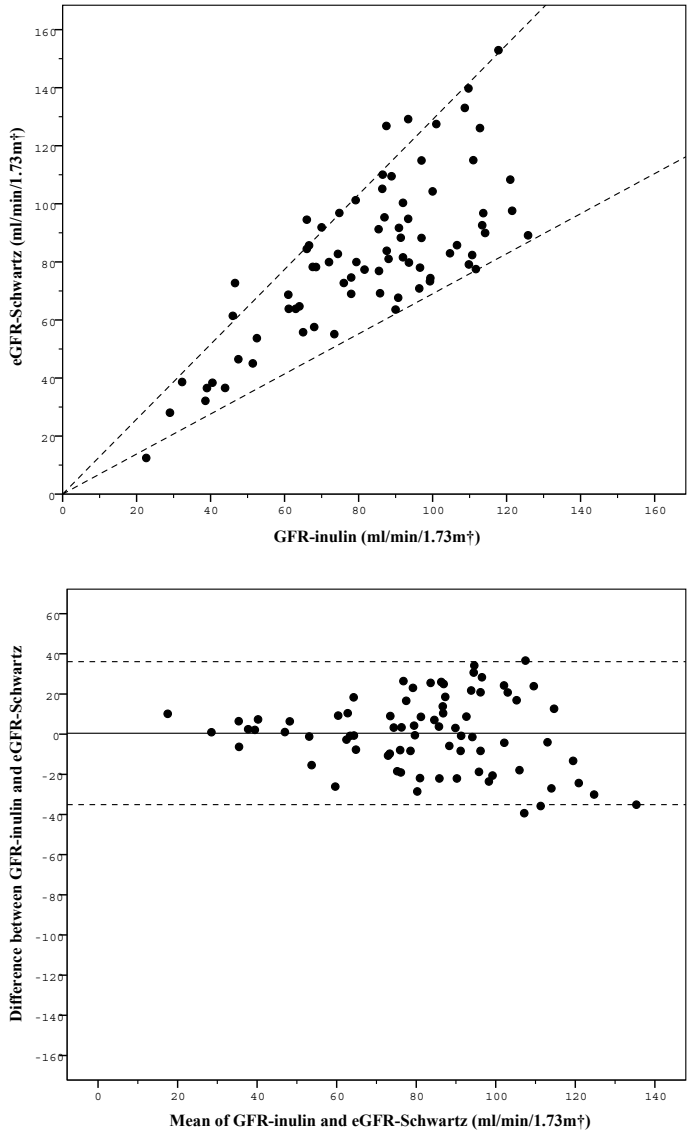

FIGURE 9.1. Accuracy of estimated GFR (eGFR) according to the Schwartz-formula (eGFR-Schwartz) versus measured GFR (GFR-inulin) in children with a solitary functioning kidney.
(**a**) Comparison between GFR-inulin and eGFR-Schwartz. The dashed lines represent the values within ±30% of GFR-inulin. (**b**) Bland-Altman plot for GFR-inulin and eGFR-Schwartz. The solid line represents the mean bias, whereas the 95% limits of agreement (i.e. mean bias ±1.96×standard deviation) are indicated by the dashed lines.

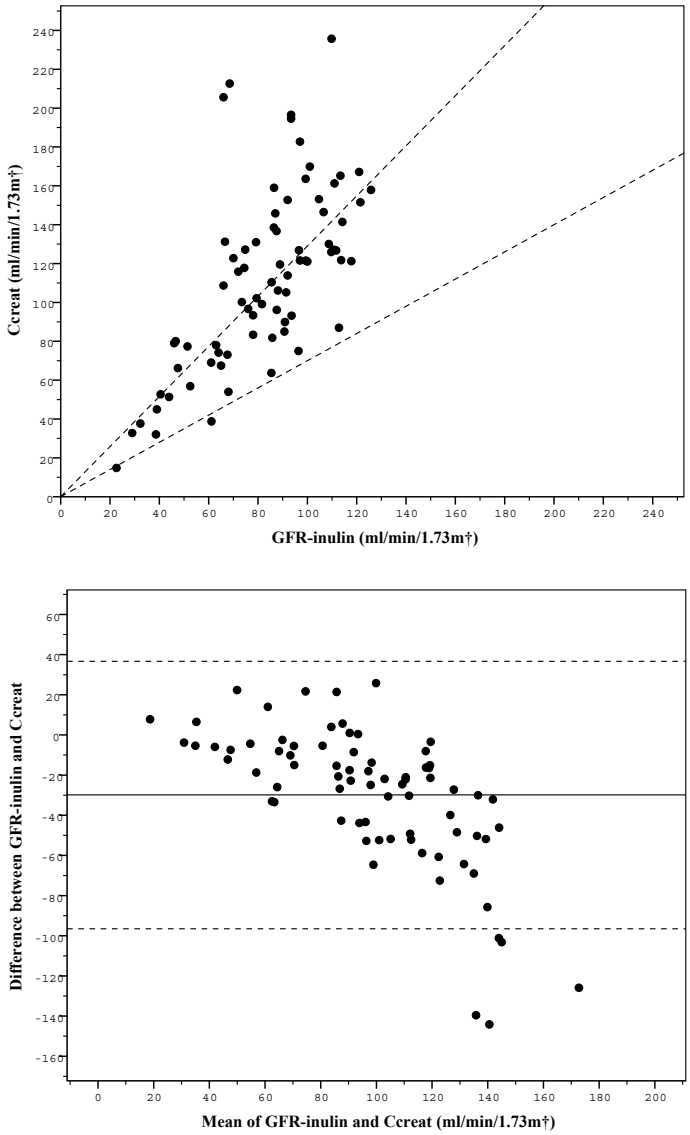

FIGURE 9.2. Accuracy of estimated GFR according to the urinary creatinine clearance (Ccreat) versus measured GFR (GFR-inulin) in children with a solitary functioning kidney.
(**a**) Comparison between GFR-inulin and Ccreat. The dashed lines represent the values within ±30% of GFR-inulin. (**b**) Bland-Altman plot for GFR-inulin and Ccreat. The solid line represents the mean bias, whereas the 95% limits of agreement (i.e. mean bias ±1.96×standard deviation) are indicated by the dashed lines.

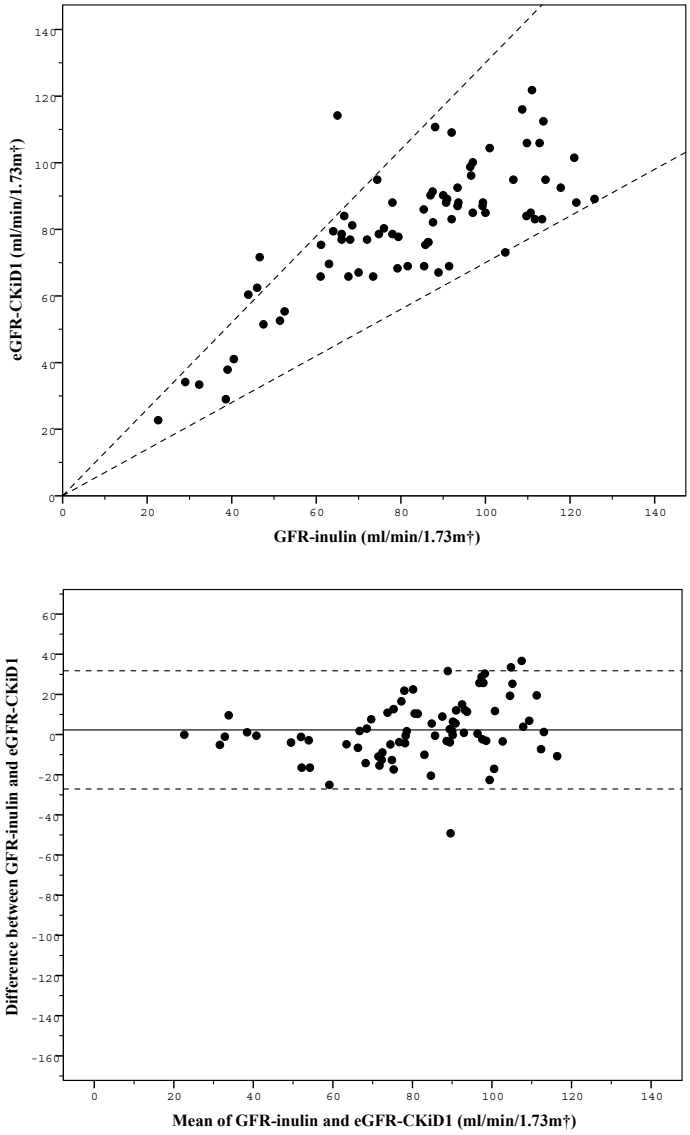

FIGURE 9.3. Accuracy of estimated GFR (eGFR) according to the serum cystatin C-based CKiD-formula (eGFR-CKiD1) versus measured GFR (GFR-inulin) in children with a solitary functioning kidney. (**a**) Comparison between GFR-inulin and eGFR-CKiD1. The dashed lines represent the values within ±30% of GFR-inulin. (**b**) Bland-Altman plot for GFR-inulin and eGFR-CKiD1. The solid line represents the mean bias, whereas the 95% limits of agreement (i.e. mean bias ±1.96×standard deviation) are indicated by the dashed lines.

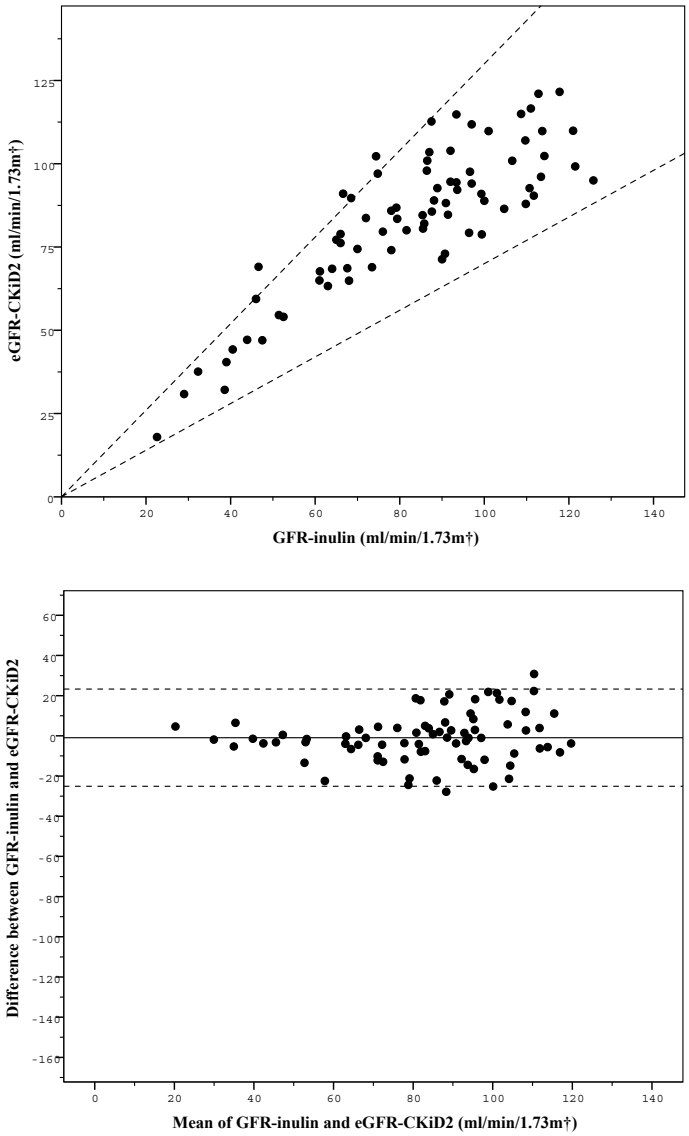

FIGURE 9.4. Accuracy of estimated GFR (eGFR) according to the combined serum cystatin C-creatinine-blood urea nitrogen-based CKiD-formula (eGFR-CKiD2) versus measured GFR (GFR-inulin) in children with a solitary functioning kidney.
(**a**) Comparison between GFR-inulin and eGFR-CKiD2. The dashed lines represent the values within ±30% of GFR-inulin. (**b**) Bland-Altman plot for GFR-inulin and eGFR-CKiD2. The solid line represents the mean bias, whereas the 95% limits of agreement (i.e. mean bias ± 1.96×standard deviation) are indicated by the dashed lines.

TABLE 9.3. Performance of estimating equations compared to GFR-inulin in children with a solitary functioning kidney.

Equation	Mean bias (ml/min per 1.73m²)*	95% LOA**	% of eGFR within ±30% of GFR inulin	% of eGFR within ±10% of GFR inulin
eGFR-Schwartz	0.4	−35.2-36.1	90%	33%
Ccreat	−29.9	−96.5-36.7	55%	14%
eGFR-Zappitelli1	−1.2	−32.9-30.5	87%	46%
eGFR-Zappitelli2	−2.8	−32.3-26.7	90%	44%
eGFR-CKiD1	2.3	−27.1-31.8	94%	46%
eGFR-CKID2	−0.9	−25.1-23.3	95%	55%

*, Bias = GFR-inulin – estimated equation. **, 95% limits of agreement = mean bias ± 1.96×standard deviation. Ccreat, urinary creatinine clearance; eGFR-CKiD1, estimated glomerular filtration rate based on serum cystatin C; eGFR-CKiD2, estimated glomerular filtration rate based on serum cystatin C, creatinine and blood urea nitrogen; eGFR-Schwartz, estimated glomerular filtration rate based on serum creatinine; eGFR-Zappitelli1, estimated glomerular filtration rate based on serum cystatin C; eGFR-Zappitelli2, estimated glomerular filtration rate based on serum cystatin C and creatinine; GFR-inulin, glomerular filtration rate based on inulin single-injection method and LOA, limits of agreement.

acquired SFK than for children with a congenital SFK. Furthermore, the acquired SFK-group showed a trend toward a lower *eGFR-Schwartz* than the congenital SFK-group ($P = 0.07$).

Comparisons between *GFR-inulin* and *eGFR-Schwartz*, *Ccreat*, *eGFR-CKiD1* and *eGFR-CKiD2* are presented in Figures 9.1-9.4. In addition, comparisons with *eGFR-Zappitelli1* and *eGFR-Zappitelli2* are displayed in Supplemental Figures 9.2 and 9.3. The performance of all equations is demonstrated by the additional Bland-Altman plots. Furthermore, the mean bias, 95% LOAs and accuracy for all estimating equations are shown in Table 9.3. On the basis of the Bland-Altman analysis, *eGFR-CKiD2* had the best performance and the smallest range in 95% LOAs (mean bias –0.9 ml/min per 1.73m², 95% LOA –25.1-23.3 ml/min per 1.73m²). The *eGFR-CKiD2* also had the best accuracy with 95% of patients within ±30% and 54% within ±10% of *GFR-inulin*, respectively. In addition, *eGFR-Schwartz* was significantly more accurate than *Ccreat* ($P = 0.01$).

Classification in CKD stage

For all patients, CKD stage based on K/DOQI guidelines is displayed in Table 9.4. Twelve (16%) patients in our cohort had CKD stage ≥3 according to *GFR-inulin*. Table 9.4 also shows misclassification in CKD stage for all estimating equations. Misclassification in CKD stage ranged from 22% of patients according to *eGFR-Zappitelli1* to 44% of patients according to *Ccreat*. Misclassification by *eGFR-Schwartz* occurred in 43% of patients. Underestimation of CKD stage (i.e. CKD stage$_{GFR\text{-}inulin}$ minus CKD stage$_{\text{estimating equation}}$ ≥1) was most frequent with *Ccreat* (n=27 [36%]) and least frequent with *eGFR-CKiD1* (n=8

TABLE 9.4. CKD stage based on K/DOQI guidelines for GFR-inulin and estimating equations in children with a solitary functioning kidney.

Equation	CKD stage 1 >90 ml/min per 1.73m^2	CKD stage 2 ≥60-<90 ml/min per 1.73m^2	CKD stage 3-5 <60 ml/min per 1.73m^2	Misclassification in CKD stage (compared to GFR-inulin)
GFR-inulin (%)	33 (43)	32 (42)	12 (16)	-
eGFR-Schwartz (%)	26 (34)	38 (49)	13 (17)	33 (43%)
Ccreat (%)	51 (66)	16 (21)	10 (13)	34 (44%)
eGFR-Zappitelli1(%)	34 (44)	34 (44)	9 (12)	17 (22%)
eGFR-Zappitelli2 (%)	32 (42)	35 (46)	10 (13)	21 (27%)
eGFR-CKiD1 (%)	21 (27)	47 (61)	9 (12)	26 (34%)
eGFR-CKiD2 (%)	32 (42)	34 (44)	11 (14)	19 (25%)

Data are presented as Number of patients (%). Misclassification is defined as the proportion of patients with an unequal CKD stage between GFR-inulin and the estimating equation.
Ccreat, urinary creatinine clearance; CKD, chronic kidney disease; eGFR-CKiD1, estimated glomerular filtration rate based on serum cystatin C; eGFR-CKiD2, estimated glomerular filtration rate based on serum cystatin C, creatinine and blood urea nitrogen; eGFR-Schwartz, estimated glomerular filtration rate based on serum creatinine; eGFR-Zappitelli1, estimated glomerular filtration rate based on serum cystatin C; eGFR-Zappitelli2, estimated glomerular filtration rate based on serum cystatin C and creatinine, and GFR-inulin, glomerular filtration rate based on inulin single-injection method.

[10%]). CKD stage was underestimated in 12 (16%) children by *eGFR-Schwartz* and in 10 (13%) children according to *eGFR-CKiD2*.

On the basis of overall performance, *eGFR-CKiD2* was the most accurate estimating equation and among the best performing equations regarding correct classification of CKD stage.

DISCUSSION

The KIMONO-study determined the precision of six commonly used estimating equations for GFR in a large cohort of children with an SFK. Our main finding is that the improved combined serum cystatin C/creatinine/BUN-based equation by Schwartz et al.[282] (*eGFR-CKiD2*) is the most precise for estimating GFR in children with an SFK. On the basis of these results, we recommend the use of *eGFR-CKiD2* in the clinical follow-up of GFR of children with different types of SFK. Furthermore, the widely used *eGFR-Schwartz* was shown to be superior to *Ccreat*. However, CKD stage was more frequently misclassified with *eGFR-Schwartz* than with the serum cystatin C equations. Therefore, *eGFR-Schwartz* is a valid alternative in children with an SFK if cystatin C is not available. Finally, although the accuracy was acceptable for most estimates, misclassification of CKD stage was identified with all estimating equations. Therefore, we emphasize that misclassification is an important caveat in estimating GFR in children with an SFK.

The concept that estimating equations might be imprecise for patients with an SFK compared with healthy two-kidney individuals has been supported by several authors.[273,284,291] Tan et al. reported that serum creatinine-based GFR estimates in adults within 86 months after living-kidney donation commonly led to misclassification of CKD stage, especially in patients older than age of 55 years.[284] According to their results, Tan et al. submit that the practice of predicting GFR from eGFR in living donors should be abandoned.[284] Pierrat et al. have compared common estimating equations with GFR measured by continuous intravenous infusion of inulin in 30 children with an acquired SFK.[273] They found an overestimation of approximately 20 ml/min per 1.73m^2 with the original Schwartz-formula,[245] which is based on age and sex. Contrary to our results, *Ccreat* in that study did not yield this difference. Unfortunately, the authors did not perform separate correlation analysis for SFK-patients and did not provide additional Bland-Altman plots, which impedes comparison with our results. The present study shows that the recalibrated *eGFR-Schwartz* accurately estimates GFR for most patients with an SFK. *Ccreat*, however, overestimates GFR and leads to underestimation of CKD stage. Bland-Altman analysis demonstrates that *Ccreat* overestimates GFR in individuals with a GFR above approximately 80 ml/min per 1.73m^2. We did not observe this in the other equations containing serum creatinine, which implies that incorrect urine collection is the most likely explanation for our finding. Because this problem is widely recognized and underlined in the K/DOQI guidelines,[270] we discourage the use of *Ccreat* to monitor GFR in patients with an SFK.

Wasilewska et al. determined serum cystatin C levels in 36 children with a congenital SFK and no other urinary tract defects.[41] Compared with a healthy control group, they found increased serum cystatin C levels in children with an SFK older than age of 12 years, whereas the original Schwartz-formula yielded normal results. In their cohort, the proportion of SFK patients with an increased serum cystatin C was similar to our study (44% versus 35%, respectively). Recently, Peco-Antic et al. demonstrated that serum cystatin C is a good predictor of renal functional reserve capacity in children with a congenital SFK and normal renal function.[44]

In our study, equations based on the combination of serum cystatin C and serum creatinine are the most precise estimates for GFR in children with an SFK. This is in accordance with data on children and adults with two kidneys.[292,293] All these results indicate that cystatin C is a promising 'early' marker for CKD in children with an SFK. Nevertheless, differences in calibration of various cystatin C assays hamper the universal use of serum cystatin C as renal marker at the moment. We therefore chose the improved CKiD and Zappitelli equations for comparison in our study because they had been derived using the DADE Behring nephelometric cystatin C assay. After an initiative by the International Federation of Clinical Chemistry, a uniform calibrator for cystatin C has been developed, which will probably improve the implementation of cystatin C as a marker for pediatric CKD.[294] In this light, it would also be interesting to compare cystatin

C with novel markers for CKD in SFK such as fibroblast growth factor 23, osteopontin and symmetric dimethylarginine.[90,267]

Significant differences between the original reports and this study should be addressed. First, although the GFR in Zappitelli and colleagues' population was similar to that in our patients with an SFK (mean GFR 74 ml/min per 1.73m^2),[279] the patients studied by Schwartz et al. had a much lower GFR (41-43 ml/min per 1.73m^2).[257,282] Because it is well known that the performance of GFR estimation equations is influenced by patient (i.e. GFR in the calibration population versus application population) and laboratory characteristics,[295] the good performance of *eGFR-Schwartz* as well as the *eGFR-CKiD* equations in our SFK population is noteworthy.

The most striking difference lies in the gold standard GFR measurement. The Zappitelli-equations were calibrated using a constant-infusion iothalamate clearance,[279] whereas the updated equations by Schwartz used a single-injection iohexol clearance.[257,282] We have used the inulin single-injection method, which has been shown to be accurate for determining true GFR in children and adults.[269,296] Inulin was measured by an enzymatic method, which has a lower sensitivity than mass spectrometry measurement and could be biased by cross-reactions with other serum metabolites such as glucose. Single-injection GFR measurements are hampered by the need for full equilibration of the tracer in the extracellular space following injection, which has to be separated from the decline in concentration reflecting glomerular filtration. This problem is particularly observed at low GFR, where late sampling is essential.[297] Indeed, Van Rossum et al. demonstrated that the inulin single-injection method used in the present study overestimated GFR by 9.7 ml/min per 1.73m^2 compared to the continuous infusion inulin clearance.[298] Taken together, these differences in methods might have influenced the performance of the various eGFR equations tested. Still, our results for both the equations by Zappitelli and by Schwartz were comparable with their original publications with respect to precision and accuracy.

In conclusion, the KIMONO-study shows that the combined serum cystatin C-serum creatinine-BUN CKiD equation estimates GFR of children with an SFK with superior precision. Hence, we recommend the use of this combined equation to monitor GFR in children with an SFK. If cystatin C measurement is not available, *eGFR-Schwartz* is an acceptable alternative and more precise than creatinine clearance, which should be abandoned. Nevertheless, we emphasize that misclassification of CKD stage remains a pitfall in estimating GFR of children with an SFK and that these patients require a gold standard GFR measurement if knowledge about the exact GFR is clinically indicated.

Acknowledgements

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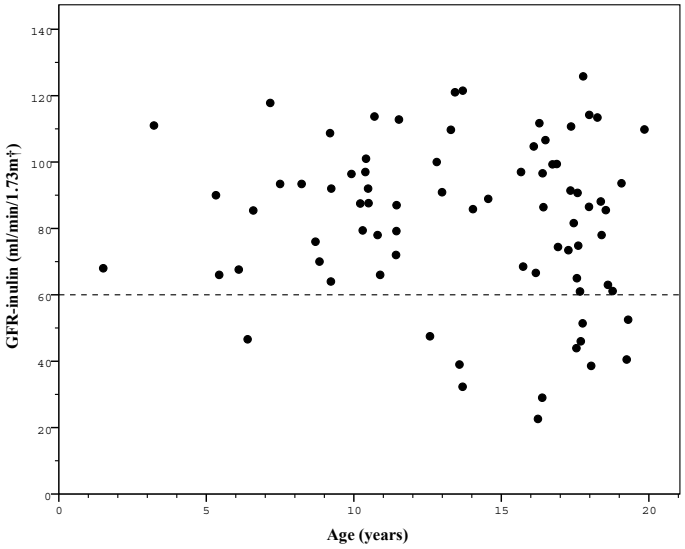
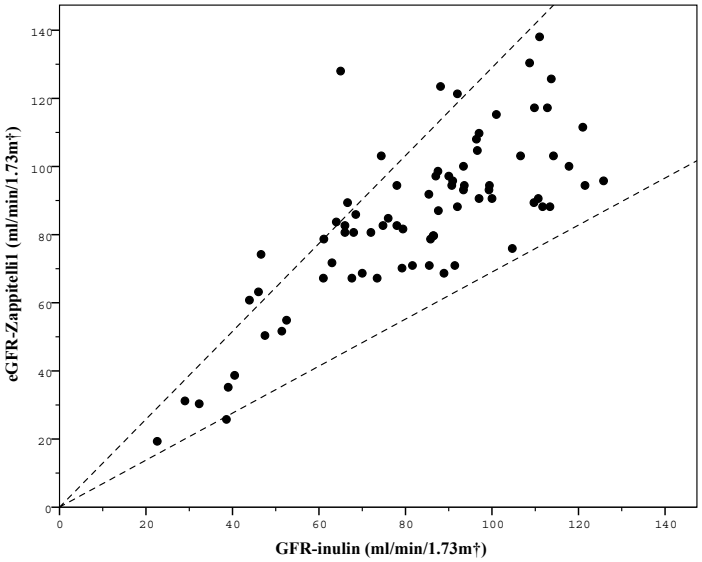
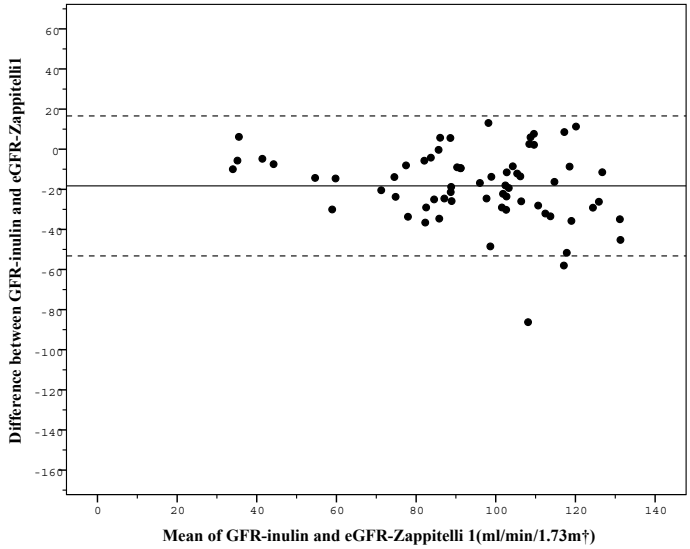
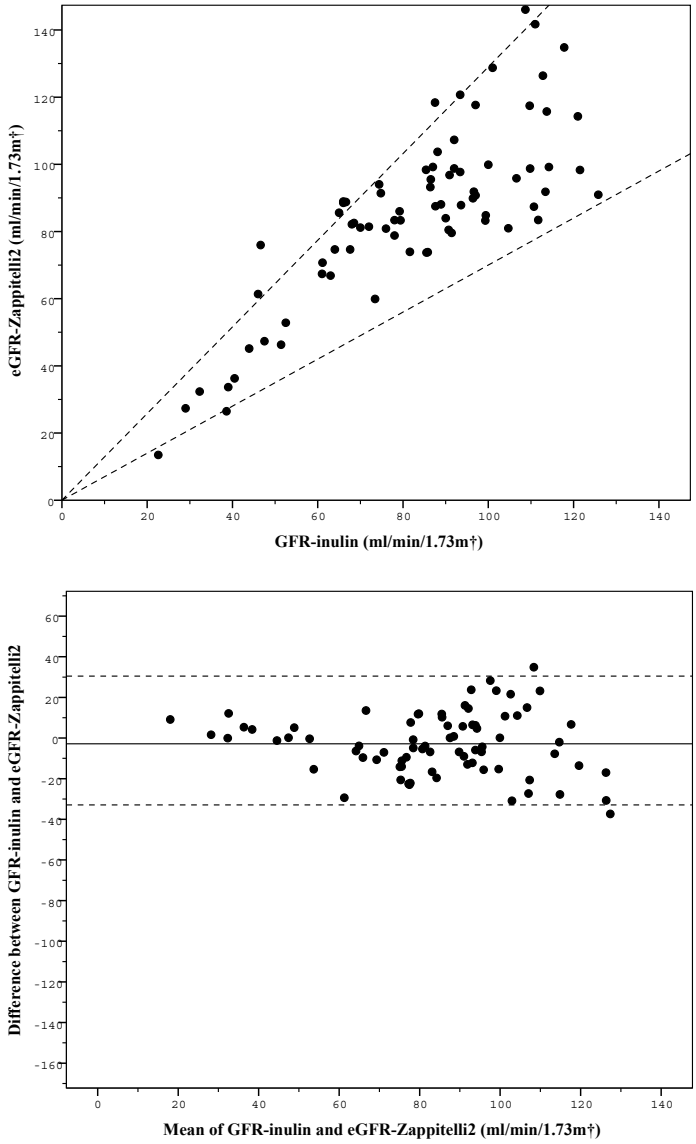


SUPPLEMENTAL FIGURE 9.1. Measured GFR (GFR-inulin) of children with a solitary functioning kidney by age. The dashed line represents K/DOQI ≥ stage 3 (60 ml/min per 1.73m^2), which is considered as chronic kidney disease.





SUPPLEMENTAL FIGURE 9.2. Accuracy of measured GFR (GFR-inulin) versus estimated GFR (eGFR) according to the cystatin C-based Zappitelli-equation (eGFR-Zappitelli1) in children with a solitary functioning kidney.
(**a**) Comparison between GFR-inulin and eGFR-Zappitteli1. The dashed lines represent the values within ±30% of GFR-inulin. (**b**) Bland-Altman plot for GFR-inulin and eGFR-Zappitelli1. The solid line represents the mean bias, whereas the 95% limits of agreement (i.e. mean bias ± 1.96×standard deviation) are indicated by the dashed lines.



SUPPLEMENTAL FIGURE 9.3. Accuracy of measured GFR (GFR-inulin) versus estimated GFR (eGFR) according to the combined serum cystatin C-creatinine-blood Zappitelli-equation (eGFR-Zappitelli2) in children with a solitary functioning kidney.

(**a**) Comparison between GFR-inulin and eGFR-Zappitteli2. The dashed lines represent the values within ±30% of GFR-inulin. (**b**) Bland-Altman plot for GFR-inulin and eGFR-Zappitelli2. The solid line represents the mean bias, whereas the 95% limits of agreement (i.e. mean bias ± 1.96×standard deviation) are indicated by the dashed lines.

Chapter 10

Ambulatory blood pressure monitoring is recommended in the clinical management of children with a solitary functioning kidney

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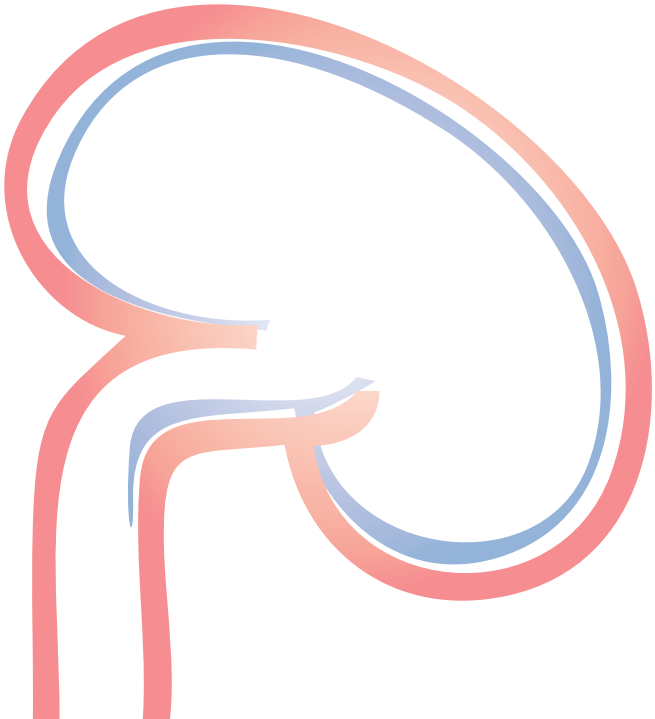


ABSTRACT

Background: Children with a solitary functioning kidney are at increased risk to develop chronic kidney disease. Hypertension may be an early indicator of renal dysfunction in these patients. We determined blood pressure (BP) profiles of children with a solitary functioning kidney by using ambulatory BP monitoring (ABPM).

Methods: To assess the occurence of (pre)hypertension, we compared ABPM to office BP measurement in 47 children with a solitary functioning kidney. None of the subjects used antihypertensive agents or had been hypertensive during previous clinical visits.

Results: Mean age of study subjects was 12.7 (±3.3) years. Hypertension was identified in 10 (21%) subjects with ABPM, whereas only 2 (4%) children were hypertensive during office BP measurement ($P < 0.01$). Fifteen (32%) children had an ABPM SD value ≥90th percentile versus 6 (13%) subjects based on office BP measurement ($P = 0.051$). Although 24h-ABPM SD scores were higher in the congenital type than in the acquired type of solitary functioning kidney ($P < 0.01$), the proportions of subjects with 24h-ABPM hypertension were similar between groups (congenital 25% versus acquired 17%; P = NS).

Conclusions: Based on ABPM, one in five children with a solitary functioning kidney has hypertension. As the majority of these subjects were not hypertensive during office BP measurements, ABPM should be considered in the clinical management of solitary functioning kidney patients.

INTRODUCTION

A substantial fraction of children with a solitary functioning kidney develops hypertension, microalbuminuria and chronic kidney disease (CKD) during childhood.[2,13] Nevertheless, the exact mechanisms underlying this renal injury are not yet fully understood.[299] Results from animal studies indicate that glomerular hyperfiltration within the remnant kidney cause hypertension and microalbuminuria at an early stage, and impairment of glomerular filtration rate (GFR) in the long term.[25,26] Hence, hypertension may be an important prognostic marker for the development of CKD in children with a solitary functioning kidney.[71] Ambulatory blood pressure monitoring (ABPM) is increasingly recognized as an indispensable modality in the diagnosis and management of hypertension during childhood and adolescence.[300] However, previous studies using ABPM in solitary functioning kidney patients have been hampered by limited patient numbers and heterogeneous study design.[38,40,130,301]

In the current study, we set out to describe blood pressure (BP) profiles of children with a solitary functioning kidney by using ABPM. Furthermore, we aim to substantiate the value of ABPM in the clinical management of solitary functioning kidney patients by comparing ABPM values to office BP measurements.

METHODS

Study subjects

All children participated in the KIMONO-study,[11] a large cohort study on the renal outcome of children with a solitary functioning kidney, and were known at the Pediatric Renal Center of the VU University Medical Center in Amsterdam, The Netherlands (recruitment period: Augustus 2012 until December 2013). Eligible subjects (n=55) were children with a solitary functioning kidney (minimum age: 6 years), who had not been treated with antihypertensive agents and were normotensive during previous clinical visits. We obtained informed consent from 53 out of 55 (96%) subjects and their parents (if applicable). The study protocol was approved by the Institutional Review Board of the VU University Medical Center. The diagnosis 'solitary functioning kidney' was based on the unilateral absence of functional renal tissue on ultrasound and/or on renal scintigraphy (<10% tracer uptake). Study subjects either had a congenital solitary functioning kidney, which is due to unilateral renal agenesis/aplasia or a multicystic dysplastic kidney, or an acquired solitary functioning kidney following uninephrectomy for congenital anomalies of the kidney and urinary tract (CAKUT; including vesicoureteral reflux), renal malignancy (i.e. Wilms' tumor) or renal vascular stenosis. As differences in the

etiology of a solitary functioning kidney may influence clinical outcome,[11] sub-analyses between solitary functioning kidney types were additionally performed.

Fifty-five patients were asked to participate in the study; informed consent was obtained from 53 (96%) subjects and their parents (if applicable). ABPM failed (i.e. <40 measurements) in 6 (11%) children, who were excluded from analyses.

Measurements

Office BP was measured with an automated oscillometric device (Dinamap Pro 100, GE Healthcare, Little Chalfont, UK), using an appropriately sized cuff. We used the mean of BP measurements to calculate SD scores for office BP, which were based on normal values for age, gender and height.[243] ABPM was performed with an oscillometric device (Spacelabs Healthcare, model 90217, Snoqualmie, WA, USA) and an appropriately sized cuff on the non-dominant arm. BP was measured every 20 minutes during the day, and every 60 minutes during the night. Based on diary information provided by the subject and his/her parents, daytime was individually defined. Only recordings with 70% of the expected number of readings, at least 20 valid daytime and seven nocturnal blood pressure measurements were included for analysis (n=47/53, 89%).[300] For each subject, ABPM SD scores based on gender and height were calculated for mean systolic and diastolic BP as well as mean arterial pressure (MAP) by using reference data of healthy children.[302] Hypertension was defined as a mean systolic and/or diastolic office BP or 24h-ABPM SD score ≥1.96 (95th percentile), whereas prehypertension was considered as a mean systolic and/or diastolic office BP or 24h-ABPM SD score ≥1.64 (90th percentile) but <1.96 (95th percentile). Also, the presence of isolated daytime and nocturnal hypertension and prehypertension was noted (ABPM SD scores ≥1.96 and ≥1.64 for day and night reference values, respectively).

Furthermore, we determined the systolic and diastolic BP load, which was defined as the proportion of measurements above the 95th percentile reference value for gender and height, and the dipping status, which was calculated as: [(daytime BP – nocturnal BP) / daytime BP] for MAP, systolic and diastolic BP.[55] Insufficient dipping was defined as a drop of less than 10% in nocturnal BP compared to daytime BP.[300]

Standard deviation (SD) scores for anthropometric data were calculated using the Fifth Dutch Growth Study data.[255] Serum creatinine (mg/dl) was measured on the day of study inclusion using an enzymatic method and GFR was estimated with the Schwartz equation (eGFR),[257] which has been shown to be reliable for children with a solitary functioning kidney.[57] Microalbuminuria was defined as a urinary albumin ≥30-300 mg/24h in a 24h urine collection on the day of study inclusion.

Renal length was determined by abdominal ultrasound and renal length SD scores were calculated using data from healthy two-kidney controls.[246,247] Compensatory hypertrophy was defined as a renal length SD score ≥1.96 (95th percentile).

Statistics

All statistical analyses were performed using IBM SPSS Statistics 22.0 (IBM Corp., Foster City, CA, USA). Continuous values are expressed as mean (±SD) in case of a normal distribution and as median (interquartile range [IQR]) in case of non-normal distribution. Assessment of normality of data was determined using normality plots and the Kolmogorov-Smirnov test. Differences were analyzed using the independent samples t-test. In case of non-normality, a non-parametric test (Mann-Whitney U test) was performed. Qualitative variables were compared using the chi-square test. Using Bland-Altman analysis,[290] we calculated the bias (i.e. mean difference between 24h-ABPM and office BP value) and the limits of agreement (LOA; mean bias ± 1.96 × SD) for systolic and diastolic BP. Accuracy was determined as the proportion of patients with an office BP measurement SD score within 30% and 10% of 24h-ABPM SD score for systolic and diastolic BP. A P-value < 0.05 was considered statistically significant in all analyses.

RESULTS

Patient characteristics

The study sample consisted of 47 patients, 28 (60%) with a congenital solitary functioning kidney and 19 (40%) with an acquired solitary functioning kidney (Table 10.1). Mean age at the time of study was 12.7 (±3.3) years (Table 10.2). The vast majority of subjects were male (68%) and Caucasian (87%). Sixteen children (34%) had additional CAKUT of the solitary functioning kidney, which encompassed vesicoureteral reflux in 9 (19%)

TABLE 10.1. Underlying phenotypes causing a solitary functioning kidney.

Type of solitary functioning kidney	Number of patients (%)
Congenital solitary functioning kidney	*28 (60)*
Unilateral renal agenesis/aplasia	16 (34)
Multicystic dysplastic kidney	12 (26)
Acquired solitary functioning kidney	*19 (40)*
Vesicoureteral reflux	14 (30)
Obstructive nephropathy	4 (9)
Wilms' tumor	1 (2)
Total	**47 (100)**

Data are presented as number of patients (%). The group of obstructive nephropathy includes pelviureteric junction obstruction (n=2), ureterovesical junction obstruction (n=1) and posterior urethral valves (n=1).

TABLE 10.2. Patient characteristics of the KIMONO-study cohort.

	All patients (N=47)	Congenital (n=28)	Acquired (n=19)	*P*-value
Male (%)	32 (68)	18 (64)	14 (74)	0.50
Age (years)	12.7 (3.3)	12.5 (3.6)	13.0 (2.9)	0.63
Height SD score	–0.05 (1.07)	–0.26 (1.10)	0.27 (0.96)	0.10
Weight SD score	0.36 (1.00)	0.29 (1.07)	0.45 (0.21)	0.59
Body Mass Index SD score	0.48 (0.96)	0.50 (0.97)	0.46 (0.97)	0.91
Renal parameters				
Serum creatinine (mg/dl)	0.61 [0.53-0.71]	0.61 [0.52-0.70]	0.62 [0.53-0.80]	0.32
eGFR (ml/min/1.73m^2)	103 (19)	104 (19)	102 (19)	0.70
Microalbuminuria (%)	7 (15)	3 (11)	4 (21)	0.33
Renal length SD score	3.40 [2.11-4.80]	3.48 [2.13-6.03]	3.14 [2.00-4.33]	0.57
Compensatory renal hypertrophy (%)	39 (83)	24 (86)	15 (79)	0.55

Data are presented as number of patients (%) or as mean (standard deviation) / median [interquartile range]. *P*-values represent differences between congenital and acquired solitary functioning kidney types. eGFR, estimated glomerular filtration rate according to the Schwartz equation and SD, standard deviation.

children, pelviureteric junction obstruction in 3 (7%) children, a congenital megaureter in 2 (4%) children and a duplex kidney in 2 (4%) children. For all patients, mean height, weight and BMI SD scores were all within -2 SD and +2 SD when compared to reference population data. Mean eGFR was normal in the congenital as well as the acquired group and all children had an eGFR >60 ml/min/1.73m^2 (eGFR-range 63-158 ml/min/1.73m^2). Microalbuminuria was identified in 7 (15%) subjects. The solitary functioning kidney demonstrated compensatory hypertrophy in the vast majority of subjects (83%). For all patient characteristics, no differences were identified between congenital and acquired solitary functioning kidney patients.

Blood pressure measurements

Office blood pressure

BP profiles of all subjects are displayed in Table 10.3. Mean office blood pressure was 114/64 (±12/±6) mmHg and mean SD scores for office BP values were both within normal range. No differences were found in systolic and diastolic office BP SD scores between solitary functioning kidney types (Table 10.3).

Ambulatory blood pressure monitoring

Mean 24h-ABPM in all subjects was 121/71 (±7/±5) mmHg. Mean 24h-ABPM SD scores for systolic and diastolic BP as well as MAP were within normal range (Table 10.3). SD scores for 24h-ABPM were higher in children with a congenital solitary functioning kidney than in children with the acquired type. Isolated daytime and nocturnal ABPM

TABLE 10.3. Blood pressure profiles of the KIMONO-study cohort.

	All patients (N=47)	Congenital (n=28)	Acquired (n=19)	*P*-value
Office BP values				
Office systolic BP SD score	0.61 (0.99)	0.76 (1.05)	0.39 (0.87)	0.21
Office diastolic BP SD score	0.06 (0.55)	0.14 (0.55)	−0.07 (0.55)	0.21
24h-ABPM values				
24h-systolic BP SD score	1.08 (0.97)	1.37 (0.87)	0.67 (0.99)	0.01
24h-diastolic BP SD score	0.63 (0.95)	0.92 (0.92)	0.22 (0.85)	0.01
24h-MAP SD score	1.03 (0.88)	1.30 (0.87)	0.63 (0.76)	< 0.01
Day systolic BP SD score	0.80 (0.97)	1.06 (0.87)	0.41 (0.99)	0.02
Day diastolic BP SD score	0.20 (0.97)	0.46 (0.95)	−0.18 (0.87)	0.02
Day MAP SD score	0.57 (0.79)	0.81 (0.77)	0.21 (0.68)	< 0.01
Night systolic BP SD score	0.97 (0.86)	1.13 (0.73)	0.74 (0.99)	0.12
Night diastolic BP SD score	0.77 (0.84)	0.91 (0.85)	0.56 (0.80)	0.16
Night MAP SD score	1.25 (0.87)	1.37 (0.81)	1.07 (0.95)	0.25
BP Load and Dipping				
24h-systolic BP load %	21.1 [10.4-38.5]	24.3 [16.7-41.1]	19.9 [4.3-35.7]	0.06
24h-diastolic BP load %	17.7 [9.0-30.0]	25.8 [10.8-30.7]	11.0 [5.1-25.0	0.03
Day systolic BP load %	22.8 [6.5-38.5]	26.8 [11.4-45.1]	16.6 [2.8-32.8]	0.04
Day diastolic BP load %	15.6 [5.2-28.0]	20.4 [7.6-29.3]	9.4 [1.0-22.3]	0.04
Night systolic BP load %	17.3 [6.2-43.7]	24.3 [7.1-43.4]	10.0 [0-44.0]	0.27
Night diastolic BP load %	20.7 [6.3-32.0]	21.9 [8.1-35.4]	14.8 [0-28.3]	0.21
Systolic BP dipping	0.11 (0.05)	0.12(0.04)	0.11 (0.05)	0.28
Diastolic BP dipping	0.17 (0.07)	0.18 (0.08)	0.16 (0.06)	0.54
MAP dipping	0.12 (0.05)	0.13 (0.06)	0.11 (0.05)	0.19
Insufficient dipping (%)	18 (38)	8 (29)	10 (53)	0.10

Data are presented as number of patients (%) or as mean (standard deviation) / median [interquartile range]. *P*-values represent differences between congenital and acquired solitary functioning kidney types. ABPM, ambulatory blood pressure monitoring; BP, blood pressure; MAP, mean arterial pressure and SD, standard deviation.

SD scores are additionally presented in Table 10.3. Children with a congenital solitary functioning kidney had higher mean SD scores for daytime systolic and diastolic BP as well as MAP than children with an acquired solitary functioning kidney type. These differences were not found in nocturnal ABPM SD scores.

Furthermore, ABPM data on BP load and dipping status are shown in Table 10.3. Compared to the acquired solitary functioning kidney type, 24h and daytime BP loads were higher in the congenital group. Insufficient dipping in systolic, diastolic and/or MAP was found in 18 (38%) children, without differences between solitary functioning kidney types.

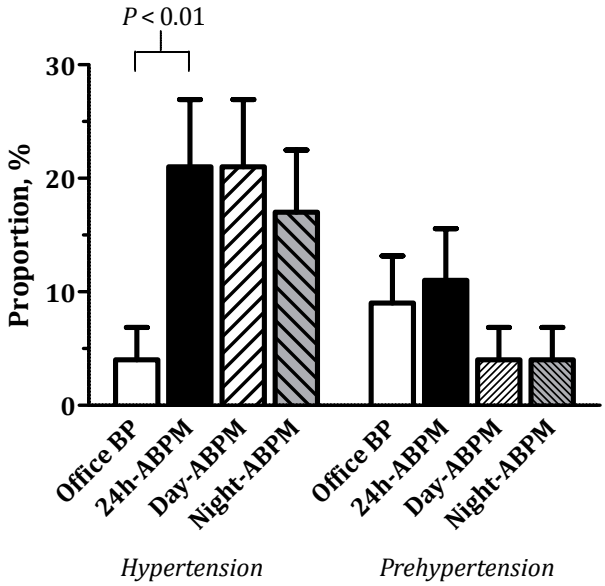


FIGURE 10.1. Hypertension and prehypertension in the KIMONO-study cohort.
Proportions of subjects with hypertension and prehypertension based on office BP and ABPM measurements. Hypertension is defined as a systolic or diastolic SD score ≥1.96 (95th percentile). Prehypertension is defined as a systolic or diastolic SD score ≥1.64 (90th percentile) but <1.96 (95th percentile). *P*-value represents the difference between 24h-ABPM and office BP measurement. ABPM, ambulatory blood pressure monitoring and BP, blood pressure.

Hypertension and prehypertension

Proportions of subjects with hypertension are presented in Figure 10.1. Based on reference data for office hypertension, 4 (9%) subjects had prehypertension and 2 (4%) had hypertension. Both patients with hypertension had a congenital solitary functioning kidney. By contrast, ABPM identified 24h-hypertension in 10 (21%) and prehypertension in 5 (11%) children. As a consequence, hypertension was more often identified by ABPM than by office BP measurement ($P = 0.005$), and both hypertensive children detected by office BP measurements were also diagnosed by ABPM. On the basis of 24h-ABPM data, 15 (32%) children with a solitary functioning kidney had a BP above the 90th percentile, compared to only 6 (13%) cases detected by office BP measurement ($P = 0.051$). There were no differences in the proportions of hypertension and prehypertension between the solitary functioning kidney types (Table 10.4).

Comparison of office blood pressure with ambulatory blood pressure monitoring

Bland-Altman analyses are presented in Supplemental Figure 10.1. Mean difference between 24h-ABPM systolic SD score and office systolic BP SD score was 0.48 (95%

LOA –1.36 to 2.32). Accuracy of office systolic BP values within 30% and 10% of systolic 24h-ABPM values was 9% and 19%, respectively. Mean difference between 24h-ABPM diastolic SD score and office diastolic BP SD score was 0.58 (95% LOA –0.92 to 2.08). Accuracy of office diastolic BP values within 30% and 10% of diastolic 24h-ABPM values was 6% and 13%, respectively.

TABLE 10.4. Hypertension and prehypertension in the KIMONO-study cohort.

	All patients (N=47)	Congenital (n=28)	Acquired (n=19)	*P*-value
Office hypertension (%)	2 (4)	2 (7)	0 (0)	0.23
Office prehypertension (%)	4 (9)	3 (11)	1 (5)	0.51
24h-hypertension (%)	10 (21)	7 (25)	3 (16)	0.45
24h-prehypertension (%)	5 (11)	4 (14)	1 (5)	0.33
Day hypertension (%)	10 (21)	8 (29)	2 (11)	0.14
Day prehypertension (%)	2 (4)	2 (7)	0 (0)	0.23
Night hypertension (%)	8 (17)	5 (18)	3 (16)	0.85
Night prehypertension (%)	2 (4)	2 (7)	0 (0)	0.23

Data are presented as number of patients (%). Hypertension is defined as a systolic or diastolic SD score ≥1.96 (95th percentile). Prehypertension is defined as a systolic or diastolic SD score ≥1.64 (90th percentile) but <1.96 (95th percentile). *P*-values represent differences between congenital and acquired solitary functioning kidney groups.

DISCUSSION

The KIMONO-study demonstrates that one in five children with a solitary functioning kidney is hypertensive when assessed by ABPM. By combining hypertension and prehypertension data, we showed that one in three children with a solitary functioning kidney has a BP above the 90th percentile. The majority of these patients were missed by office BP measurement. In addition, insufficient nocturnal BP dipping, which is associated with a poor cardiovascular outcome,[303] was identified in an additional 18 (38%) subjects. Considering the link between (pre)hypertension, cardiovascular disease and CKD,[304] our findings indicate that children with a solitary functioning kidney are at risk for an impaired clinical outcome.

Results from our study are in line with the increasing concerns about the prognosis of individuals with solitary functioning kidneys.[49,299] A longitudinal clinical study has previously shown that 20-50% of adults with a solitary functioning kidney from childhood develop end-stage renal disease by the age of 30 years.[2] Although this study describes a selected group of patients and its conclusions can therefore not be generalized to all soli-

tary functioning kidney patients, data from animal studies as well as human studies have suggested a poorer renal outcome in these children.[25,27,36,37,39] Landmark studies by the group of Brenner have hypothesized that glomerular hyperfiltration following renal mass reduction is the pathophysiological driver for this impaired prognosis.[25] Before developing CKD, animals with renal mass reduction were hypertensive and proteinuric. In a large retrospective study of 407 children with both solitary functioning kidney types,[13] we have recently shown that 50% of subjects had hypertension (determined by office BP), microalbuminuria or an impaired eGFR at a median age of 15 years. These findings indicate that hypertension is a cardinal indicator in the clinical outcome of these patients.

Our findings indicate that ABPM is of additional value in the clinical management of hypertension in solitary functioning kidney patients. In a cohort of children who were normotensive during clinical visits prior to this study, we demonstrate that ABPM identifies hypertension in an additional 17% of subjects when compared to office BP measurement (i.e. masked hypertension). Both subjects who were hypertensive during office BP measurements also had hypertension with ABPM. Moreover, Bland-Altman and accuracy analyses showed that office BP measurement is inaccurate when compared to 24h-ABPM. Furthermore, ABPM provides clinicians with additional information on white coat hypertension, which was absent in our cohort, blood pressure load and dipping status as well as isolated daytime and nocturnal hypertension.[300] On the basis of these findings, ABPM should be part of the clinical management of children with a solitary functioning kidney. We recommend screening for hypertension with ABPM once every year as this will allow for timely interventions,[299] which mainly constitutes angiotensin converting enzyme (ACE)-inhibition and angiotensin receptor blockers (ARBs). Indeed, early intervention with ACE-inhibition and stringent blood pressure control (i.e. MAP <50th percentile) has previously shown to improve the clinical outcome of children with renal hypodysplasia.[52]

Another important finding of this study are the higher ABPM SD scores in the congenital compared to the acquired type of a solitary functioning kidney. Interestingly, these increased values were only found in 24h and daytime BP values. Our results are remarkable, as previous studies have never reported differences in BP between solitary functioning kidney types.[11,35,38] The observed differences in mean ABPM values do not translate into differences in the proportions of study subjects with hypertension or prehypertension between both groups. As 24h and daytime BP values are of less predictive value because they are more subjected to movement artifacts than nocturnal measurements[300] and the lack of statistical significance between night SD scores is most likely due to the small sample size (type II error), these findings require further investigation.

Previous studies on ABPM in children with a solitary functioning kidney reported a wide range in the detection of hypertension (from 0-32%, respectively).[38,40,130,301] However, none of these studies compared ABPM to office BP measurements. In addition, the re-

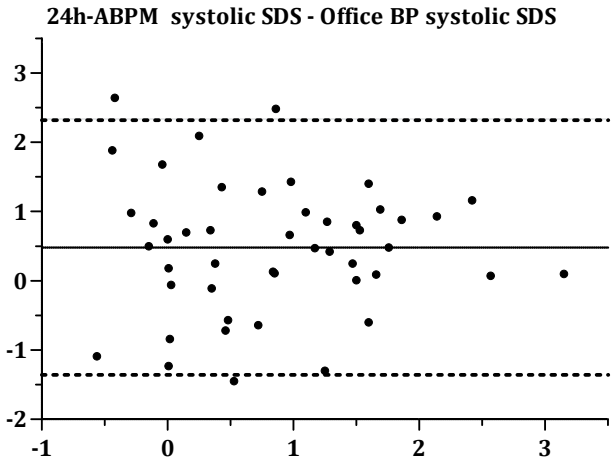
ported studies were different in the solitary functioning kidney types studied, reference values and the ABPM device used. We calculated ABPM SD scores based on the recently updated American Heart Association recommendations.[305] Furthermore, we used an oscillometric device that has been validated in children, and is relatively small and light to minimize patient discomfort. Finally, recent studies have investigated the association between hypertension and compensatory renal hypertrophy[38] and microalbuminuria[301] in children with a solitary functioning kidney. Although our sample size was too small to investigate such associations, studies on the identification of risk factors for hypertension and CKD such as insufficient renal hypertrophy, birth weight, microalbuminuria and the presence of additional CAKUT are highly needed.[13]

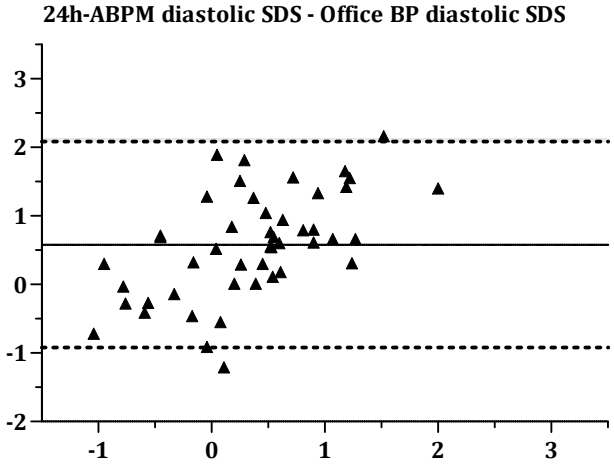
An important strength of our study is the clinically well-defined cohort of children with a solitary functioning kidney. None of the patients had CKD stage 3 or higher, whereas 15% of patients had microalbuminuria at the time of study. Our study has several limitations. As the study site is a large referral center, our results may be subjected to ascertainment bias and consequently overestimate the proportion of hypertension in our study cohort. In contrast, ABPM was only performed in individuals that had been normotensive at preceding outpatient visits, thereby excluding solitary functioning kidney patients with hypertension from this study. Compared to our previous KIMONO-studies,[13] this has by definition reduced the proportion of hypertension found by ABPM in the current cohort. Notably, a recent report indicated that increased ABPM values compared to office BP measurements are a relatively more frequent finding in children compared to adults.[306] Furthermore, we did not perform ABPM studies in age-matched healthy controls but based our SD scores on normative population data as presented in the most recent recommendations of the American Heart Association.[302,305] Although these reference values are well established, ABPM SD scores of healthy controls could have been different in our hands. Finally, the generalizability of our findings may be limited due to the relatively small number of subjects. However, we feel that our results provide a strong rationale to perform large longitudinal ABPM studies in this specific patient group.

In summary, the KIMONO-study demonstrates that one in five children with a solitary functioning kidney exhibited hypertension based on ABPM. Moreover, the majority of these subjects were missed with office BP measurements. As hypertension is likely to be a prognostic marker in the development of CKD in these children, our findings underline the cardinal role of ABPM in the clinical management of solitary functioning kidney patients.

Acknowledgements

We thank all patients and their parents for participating in the KIMONO-study, and Monique Koot for her excellent administrative assistance.





SUPPLEMENTAL FIGURE 10.1. Bland-Altman plots for (**a**), systolic blood pressure and (**b**), diastolic blood pressure.

Circles represent systolic BP SD scores, whereas diastolic BP SD scores are displayed by triangles. Mean bias (difference between 24h-ABPM and office BP SD scores) is depicted by the solid line and the 95% limits of agreement (i.e. mean bias ± 1.96 × SD) is shown by the dashed lines. ABPM, ambulatory blood pressure monitoring and BP, blood pressure.

Chapter 11

Recessive mutations in CAKUT and VACTERL association

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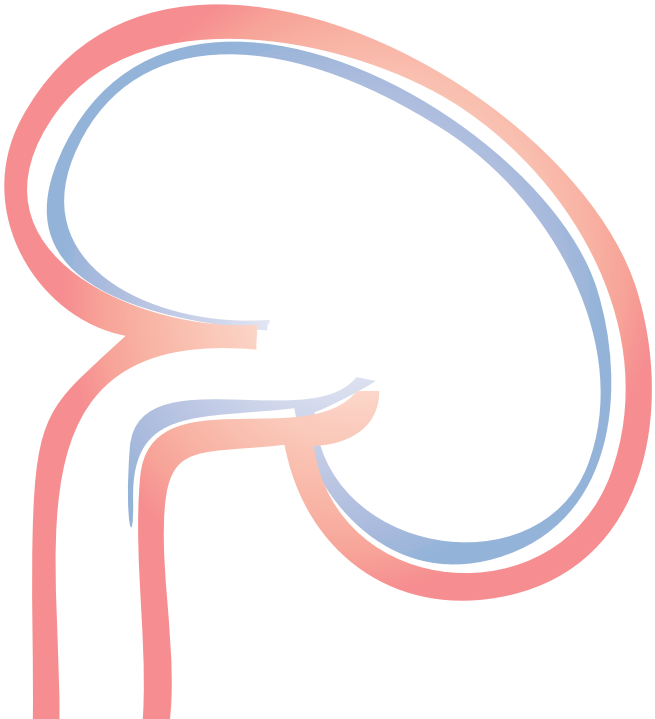

ABSTRACT

Understanding the genetic makeup underlying congenital anomalies of the kidney and urinary tract (CAKUT) is of primary importance to improve diagnosis, stratify risk for later-onset complications, and develop therapeutic strategies. Saisawat et al.[309] used homozygosity mapping with next-generation sequencing to identify recessive mutations in *TRAP1* in families with isolated CAKUT and with VACTERL association. This study points to a novel player in kidney development, possibly affecting apoptosis and endoplasmic reticulum stress signaling.

Congenital anomalies of the kidney and urinary tract (CAKUT) represent 23% of all identified birth defects and are the cause of end-stage renal disease (ESRD) in as many as 50% of pediatric cases.[307] In the adult population, the fraction of ESRD attributable to CAKUT has been estimated around 7%.[82]

CAKUT encompass a broad spectrum of phenotypes ranging from parenchymal defects such as renal agenesis and hypodysplasia, to malformations of the urinary tract, such as ureteropelvic junction obstruction, megaureter, and vesicoureteral reflux. These phenotypes result from a perturbation in normal kidney development, a process that is largely governed by a series of reciprocal interactions between the ureteric bud and the metanephric mesenchyme. Many growth factors, expressed in either the metanephric mesenchyme, the ureteric bud, or both, have been implicated in nephrogenesis, providing evidence for high genetic heterogeneity for urinary tract malformations.[7]

The genetic architecture of CAKUT is complex, with both point mutations and structural variants contributing to this trait. In about 90% of cases CAKUT occur as sporadic disease, suggesting that *de novo* dominant mutations or recessive inheritance may account for a significant proportion of these cases. In a survey on 522 cases with renal hypodysplasia, we demonstrated that the disease was caused by rare, often unique, heterozygous copy-number variations in almost 20% of the cases.[21] In about 10% of cases the disease occurs in families with autosomal dominant (more frequent) and recessive (less frequent) patterns of inheritance.[12,308] Despite familial aggregation of the disease, gene discovery has been slow for this trait, because of the extreme genetic heterogeneity, the incomplete penetrance of disease, and the usually small pedigree size. As a result, most of the mutations identified so far affect genes implicated in syndromic forms of disease, which are recognizable because of their extra-renal phenotypes.[12] Few genes have been implicated in isolated forms of CAKUT in a sizeable fraction of patients, and include *HNF1B*, *PAX2* and, more recently identified, *DSTYK*,[16] but the vast majority of causes for CAKUT are still unknown.

Saisawat et al.[309] now identify recessive mutations in TNF receptor-associated protein 1 (*TRAP1*) in individuals with CAKUT and VACTERL association, a well-defined variable phenotype encompassing Vertebral defects, Anorectal malformations, Cardiac defects, TracheoEsophageal fistula/atresia, Renal malformations and Limb defects.

In this elegant study, the authors hypothesized that CAKUT and CAKUT in VACTERL association are caused by rare recessive mutations and studied a total of 677 CAKUT patients and 301 patients with VACTERL association. By homozygosity mapping coupled with whole exome sequencing in two families, one with CAKUT and one with CAKUT and VACTERL association, they identified the same mutation, p.R469H, in homozygous state, in *TRAP1*. Interestingly, this variant, present in controls at a frequency of 0.9%, was shown to be of ancestral European origin by haplotype analysis. To validate the results, the authors screened the *TRAP1* coding region for mutations in an additional

675 CAKUT and 300 VACTERL patients using an original high-throughput and low-cost approach based on barcoded multiplex PCR followed by next-generation sequencing. This strategy allows fast and high-coverage sequencing for large cohorts of patients in a single experiment. They detected six additional mutations in *TRAP1* in three unrelated families. Importantly, one of these mutations was a frameshift truncating mutation (p.R46fs*75), suggesting a loss of function mechanism of disease pathogenesis, consistent with the recessive inheritance. All other mutations were missense variants at conserved amino acid residues that were rare or absent in publicly available databases. Since some of these variants were found at low frequency in controls, the authors sought to investigate if there were individuals carrying rare variants on both *TRAP1* alleles. The authors nicely demonstrated that none of 800 patients with nephronophthisis (used here as controls), sequenced with the same technology as the cases, harbored deleterious mutations in either homozygous or compound heterozygous state. *TRAP1* encodes for tumor necrosis factor receptor associated protein 1, a heat shock protein 90 (Hsp90)-related mitochondrial chaperone. The authors do not provide functional data but they show that Trap1 is expressed in renal epithelia of the developing mouse kidney and in the adult rat kidney, especially in the renal cortex and medulla.

In summary, Saisawat and colleagues[309] identified *TRAP1* recessive mutations in about 0.5% of patients with CAKUT and CAKUT in VACTERL association. While the fraction of disease attributable to variants in *TRAP1* seems very small, it does not come as a surprise, given the high genetic heterogeneity of kidney and urinary tract malformations. In a recent study we identified 72 different copy-number variations in 87 out of 522 patients, indicating that the genetic heterogeneity of this trait might be even higher than expected and that each susceptibility gene is likely to account for the disease in a very small proportion of cases.[21] As a confirmation that CAKUT is the disease of "hundreds of genes", point mutations in the three genes most commonly associated with this disease, *HNF1B*, *PAX2*, and *DSTYK*, are identified in 10-15% of patients with developmental diseases of the kidney and urinary tract.[16,17] Given the epidemiology of CAKUT, recessive genes are likely to be even rarer, making this discovery more significant.

The presence of deleterious variants in *TRAP1* in control individuals poses the question whether the mutations identified here are polymorphisms or true disease-causing alleles. Although definitive proof of causation will require replication in larger cohorts and generation of animal models of disease, the absence of *TRAP1* homozygous or compound heterozygous deleterious (recessive) variants in 800 patients with nephronophthisis makes the case for a causal link stronger. It is in fact well known that recessive mutations are less subject to purifying selection than dominant mutations and can remain fixed in populations at low frequency.

The role of *TRAP1* mutations in determining CAKUT and CAKUT in VACTERL association is presently unknown. *TRAP1* is an evolutionary conserved mitochon-

drial chaperone of the Hsp90 gene family,[310] members of which have been implicated as indispensable regulators of protein folding quality control. Inhibition of TRAP1 and mitochondrial Hsp90 ATPase results in activation of apoptosis, up-regulation of a stress response gene signature and compensatory autophagy. The functions of TRAP1 have been broadly linked to the development of cancer and Parkinson's disease, in which overexpression of *TRAP1* modulates oxidative stress responses and antagonizes reactive oxygen species-mediated cellular damage.[310] However, TRAP1 and other Hsp90 members have also been implicated in normal and abnormal development in various animal models. For example, inhibition of Hsp90 by geldanamycin decreases developmental stability in zebrafish.[311] Interestingly, Zhu et al. found that *TRAP1* is involved in abnormal limb development in mice, a feature that is often identified in VACTERL association.[312] It is therefore conceivable that mutations that alter one or more of the functions of *TRAP1* in a time- and cell-specific manner during embryonic development can give rise to multiple organs malformations including CAKUT and other features of VACTERL association (Figure 11.1).

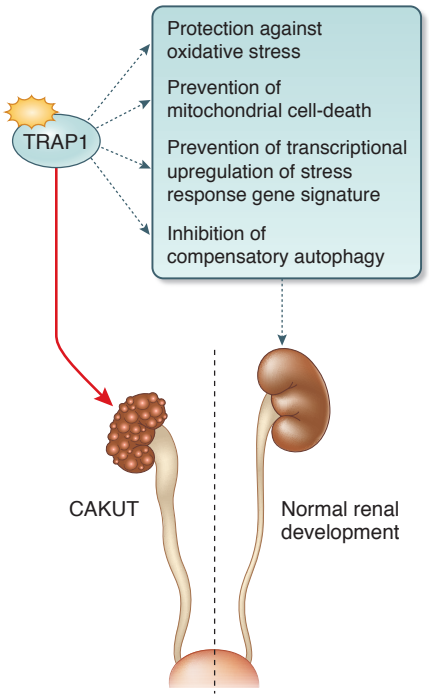

FIGURE 11.1. Potential pathways of *TRAP1* mutations leading to CAKUT.
Mutations in tumor necrosis factor receptor-associated protein 1 (*TRAP1*) may lead to reactive oxygen species-mediated apoptosis, up-regulation of a stress response gene signature, and autophagy and subsequently perturb renal development, causing congenital anomalies of the kidney and urinary tract (CAKUT)

Congenital kidney and urinary tract malformations are frequently observed in the general population, they are the major cause of pediatric end-stage renal disease requiring dialysis or transplantation, and they predispose children to severe cardiovascular complications later in life. Moreover, given the significant improvement in neonatal and pediatric care, more of these children are surviving to reach adulthood, suggesting, first, that the proportion of this patients in the adult ESRD population will increase and, second, that more deleterious mutations, normally subjected to purifying selections, will be transmitted through generations.

All these observations call for a major effort in identifying the molecular defects underlying CAKUT in order to improve diagnosis, to conduct appropriate genetic counseling and help parental planning, to devise tools for risk stratification for renal and extra-renal diseases that develop later in life, and to develop individualized treatment strategies. Given the complex genetic architecture of the disease, identification of disease-causing mutations in CAKUT has been proven difficult. The recent technological advancements in genotyping and sequencing methodologies now allow us to rapidly identify candidate variants (either point mutations or copy-number variations) at a genome-wide level in single families or even in single individuals. The challenge now is to find independent *bona fide* mutations in order to declare genetic causation and proceed to functional studies in animal models. To tackle this difficult task, it is becoming obvious that large collaborations between investigators are warranted in order to cross-validate and replicate findings coming from exome sequencing or copy-number variation studies, before we think of moving again to the next available technology.

Chapter 12

Copy-number analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney from the KIMONO-study

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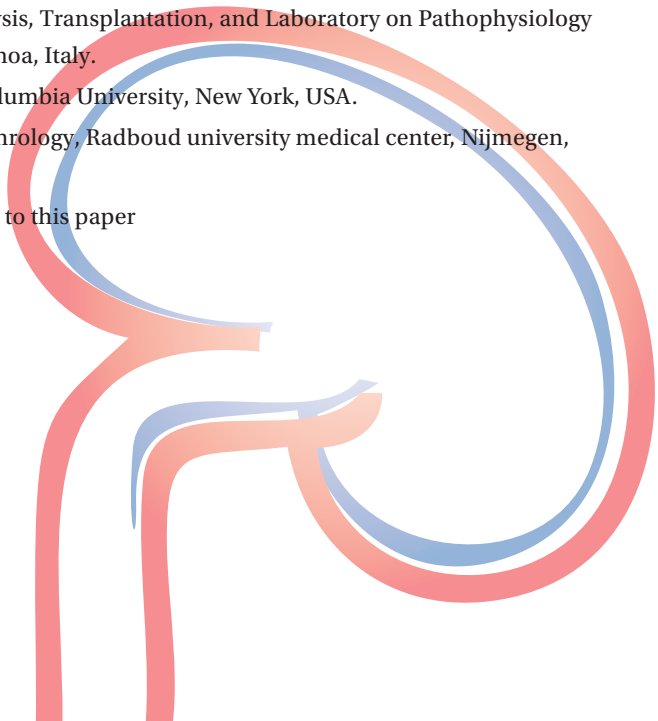
* MV and KV contributed equally to this paper

Submitted



ABSTRACT

Copy-number variations (CNVs) have been associated with different developmental phenotypes and represent a major cause of congenital anomalies of the kidney and urinary tract (CAKUT). Since rare CNVs are often large and contain multiple genes, the inference of causality is relatively simple. Nevertheless, the identification of the underlying genetic drivers has proven to be difficult. Here we investigated the role of rare CNVs in 80 children from the KIMONO-study cohort (mostly affected by renal hypodysplasia) for which pathogenic mutations in the three most common genes implicated in renal malformations (*HNF1B*, *PAX2*, and *DSTYK*) were excluded. In total, we identified 13 known or novel genomic disorders in 11/80 patients (14%) that were absent or extremely rare in 23,362 population controls. To identify the most likely genetic drivers for the CAKUT phenotype underlying these rare CNVs, we used a systematic *in silico* approach based on frequency in large datasets of controls, annotations with publicly available databases for developmental diseases, tolerance and haploinsufficiency scores, and gene expression profile in the developing kidney and urinary tract. Using this strategy we identified five high-priority novel candidate genes for CAKUT. All candidate genes showed specific expression in the mouse developing urinary tract. Among these genes, we propose *DLG1* and *KIF12* as most likely novel susceptibility genes for CAKUT in humans. Our study confirms the significant role of genomic imbalances in the determination of kidney developmental phenotypes and defines a systematic strategy to identify genetic drivers underlying rare CNVs.

INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) are the leading cause of end-stage renal disease in childhood.[1] A solitary functioning kidney represents a frequent phenotype among the spectrum of CAKUT, and may profoundly impact long-term clinical outcome.[2,13] Phenotypes underlying a solitary functioning kidney of congenital origin include conditions causing the primary absence of normally developed unilateral renal parenchyma,[299] such as unilateral renal agenesis (URA), multicystic dysplastic kidney (MCDK), and renal hypodysplasia (RHD), or conditions leading to recurrent infections or severe hydronephrosis that require nephrectomy in childhood, such as vesicoureteral reflux (VUR), ureteropelvic junction obstruction (UPJO) and congenital megaureter.[35]

Genetic factors play a major role in the pathogenesis of CAKUT in mammals.[7,12,15,313] Familial aggregation is identified in approximately 14% of cases,[114] with different modes of inheritance.[308,309,314,315] Both point mutations and structural variants are implicated in disease determination.[21,313,316] Approximately 10-20% of isolated CAKUT-phenotypes underlying a solitary functioning kidney are caused by single-gene defects[17,18,309] with pathogenic mutations in *HNF1B* (MIM189907),[317] *PAX2* (MIM167409),[318] and *DSTYK* (MIM612666)[16] among the most frequently implicated. Copy-number variations (CNVs), defined as gain or loss of germ line DNA of the size ranging from 1 kilobase (kb) to several megabases (Mb),[319] have been associated with multiple human phenotypes, including neurodevelopmental diseases (intellectual disability, autism, schizophrenia, epilepsy), cardiac defects, lung disease, craniofacial malformations, and others.[320-324] We recently demonstrated that rare genic CNVs represent a major molecular determinant of kidney malformations, accounting for up to 17% of patients with RHD.[21] The identification of rare deletions or duplications that affect multiple genes can establish a genetic diagnosis, therefore improving patient care and genetic counseling. Nevertheless, given that CNVs usually affect the dosage of multiple genes, the interpretation of the molecular defect that leads to the observed phenotype is rather challenging. As a result, the identification of the major genetic drivers underlying such events has proven difficult.[325]

In this study, we investigated the role of rare CNVs in children with a solitary functioning kidney derived from the KIMONO (KIdney of MONofunctional Origin)-study in whom pathogenic mutations in *HNF1B*, *PAX2*, and *DSTYK* were excluded. To identify the most likely drivers for the CAKUT phenotype in carriers of pathogenic CNVs, we performed a systematic *in silico* approach using bioinformatic resources and expression profiling in the developing mouse kidney. By using this approach, we identified five high-priority genes and we propose *DLG1* and *KIF12* as novel candidate genes for human kidney and urinary tract malformations. This study provides an analytical pipe-

line to help interpret the functional consequence of CNVs and provides a list of novel candidate CAKUT susceptibility genes for follow-up validation and functional studies.

RESULTS

Patient characteristics

The KIMONO-GENE cohort included 80 patients (Supplementary Table 12.1), of whom 54 (68%) patients were males. The vast majority of subjects were Caucasian. Primary kidney parenchyma defects (URA, MCDK, and RHD) were present in 66 (83%) children. Thirty-seven (46%) subjects had a solitary functioning kidney with additional CAKUT such as VUR, UPJO or a megaureter. Additional clinical parameters are presented in Supplementary Table 12.2.

Exclusion of mutations in *HNF1B*, *PAX2*, and *DSTYK*

Individuals were screened for point mutations in the coding regions of *HNF1B*, *PAX2* and *DSTYK* by Sanger sequencing as previously described.[16,18] No pathogenic mutations were identified. An overview of all identified single nucleotide variants is presented in Supplementary Table 12.3.

Genomic disorders are frequent in the KIMONO-GENE cohort

CNV analysis in the 80 children from the KIMONO-GENE cohort identified 118 large CNVs (defined as CNV size >100 kb; 1.48 CNVs per case; Supplementary Table 12.4). Median large CNV-size was 174,888 bp [IQR 136,364-263,382 bp]. The majority of these large CNVs were duplications (n=68, 58% versus deletions: n=50, 42%). Annotation of large (≥100 kb) rare (<1:1,000) CNVs identified 5/80 individuals (6%) that carried 6 genomic imbalances with significant overlap with known genomic disorders (Table 12.1). As expected, a substantial proportion (50%) of these subjects had extra-renal anomalies. Among these, one subject with MCDK, VUR, cleft palate and attention deficit disorder carried the 3q29 microdeletion syndrome spanning 1.61 Mb, which disrupted multiple genes and was absent in 23,362 controls, and another individual with MCDK and ureterocele harbored a 570 kb duplication at the *PKD1* locus on chromosome 16p13.3, which was also absent in controls. We then restricted our search for pathogenic CNVs to very rare events (frequency <1:4,000) and identified seven independent novel rare CNVs in 7/80 cases (9%; Table 12.2), including three single gene deletions on chromosome 10q21.1, 13q21.32, and Xq12. The Xq12 deletion was found in a male subject with congenital VUR, indicating complete loss-of-function. In total, 4 out of 11 (37%) patients with potentially pathogenic CNVs exhibited neurodevelopmental defects. Consistent with prior reports,[21,326] 2/80 individuals (2.5%) were found to carry more than one large

rare CNV. Ten intergenic CNVs that were absent in controls are reported in Supplementary Table 12.5. In total, we identified 13 known or novel genomic disorders in 11 (14%) of patients.

Systematic *in silico* approach identifies potential genetic drivers for 6 CNV phenotypes

In order to define the candidate genetic drivers of the CNV phenotype, we established a systematic *in silico* approach by using bioinformatic resources (see Figure 12.1 and methods below). The 13 identified large, rare, genic CNVs included a total of 151 genes. All genes underlying the CNVs overlapping with known genomic disorders were retained (n=119). Novel genomic disorders were annotated against the International Standards for Cytogenomic Arrays Consortium (ISCA) database and Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources Consortium (DECIPHER) to select CNVs that overlapped with variants of likely pathogenic significance (Supplementary Table 12.6 and Supplemental Figure 12.1). After alignment, we discarded the deletion on chromosome 1q44 and the duplication on chromosome 3p26, as both CNVs showed significant overlap with benign variants (Supplementary Figure 12.1). In total, 137 genes were assessed for their potential pathogenic role. We cross-annotated our genes with the Exome Variant Server (EVS) and included all deleted genes that carry truncating mutations in <1:1,000 individuals and all duplicated genes that carry deleterious missense variants in <5:1,000 individuals (Supplementary Table 12.7 and 12.8, respectively). The resulting 32 genes were subsequently interrogated for the haploinsufficiency logarithm of the odds (HI-LOD) score[327] and the residual variation intolerance (RVI)-score[328] (Supplementary Tables 12.9 [whole deletions] and 12.10-11 [prioritized genes], respectively). We defined a HI-LOD score ≥2 and the 10th percentile of the calculated RVI-score as threshold values for genes that are more likely to result in a phenotype when mutated. Using these criteria we identified two genes. We included all single-gene CNVs in our systematic approach, as these variants may directly point to the genetic defect (n=2). Finally, we included all genes that are implicated in renal disease and genes in which disruption in murine orthologs leads to kidney development defects (n=2, from which 1 overlapped with our previous criteria). The resulting list of high-priority candidate genes (n=5) included: *DLG1* (MIM601014), *EDA2R* (MIM300276), *KIF12* (MIM611278), *PCDH9* (MIM605514), and *TRAF7* (MIM606692) (Table 12.3). Consistently, rare truncating variants are extremely rare in this genes (Supplementary Table 12.12), suggesting that deleterious mutations have been eliminated by purifying selection.

Expression profiles of candidate genes for CAKUT

We evaluated gene expression profiles in the developing mouse kidney for all high-priority genes by using GenitoUrinary Development Molecular Anatomy Project (GUDMAP) and Genepaint databases. According to these databases, all five genes were expressed in the developing mouse kidney and urinary tract. We subsequently performed localization studies using immunofluorescence in the developing embryonic mouse kidney at embryonic day E14.5. As expected from data implicating *Dlg1* in urinary tract malformations in mice,[329] this gene was heavily expressed in ureteric bud (UB) and metanephric mesenchyme (MM) structure (Supplementary Figure 12.2A-B). Figure 12.2 demonstrates strong expression of Kif12 in the branching UB and, to a lesser extent, in the MM and developing nephrons. Expression profiles of *Eda2r*, *Pcdh9*, and *Traf7* are additionally presented in Supplementary Figure 12.2 and show moderate expression levels in the developing kidney.

DISCUSSION

CAKUT has an overall prevalence of about 1%[330,331] and accounts for 40-50% of pediatric cases of chronic kidney disease.[81,82] Establishing an early molecular diagnosis in such patients may therefore significantly affect risk stratification for complications that develop later in life, therapeutic trajectories, and clinical outcome. In the current study we identified rare known or novel genomic disorders in 14% of children affected by a solitary functioning kidney without point mutations in common CAKUT susceptibility genes, confirming that human kidney and urinary tract development is particularly sensitive to gene dosage. We have previously shown that the large majority of CNVs found in kidney malformations are also implicated in developmental delay, autism, schizophrenia and other neurocognitive phenotypes.[21] In this study, clinical data confirmed the presence of neurodevelopmental defects (e.g. intellectual disability, autism, and attention deficit hyperactivity disorder) in 37% of patients with potentially pathogenic CNVs. Identified genomic imbalances in CAKUT, which can already be visualized by prenatal ultrasound, may therefore function as a sentinel for neurocognitive defects before they become clinically evident. Ideally, this early diagnosis will not only improve renal outcome in such patients, but also lead to tailor-made familial counseling and early intervention to ameliorate overall clinical outcome.

In the recent decade there has been tremendous improvement in our understanding of structural variations in the determination of both Mendelian and complex diseases. In fact, genomic disorders have been implicated in a wide variety of developmental phenotypes such as schizophrenia, autism, epilepsy, intellectual disability, cardiac malformations, craniofacial malformations, CAKUT, and others.[21,320-324] While these studies

TABLE 12.1. Known diagnostic genomic disorders identified in the KIMONO-GENE cohort.

Chr	CNV type	Start (Mb)	End (Mb)	Size (Mb)	Syndrome	N. of genes	KIMONO cohort (n=80)	Controls (n=23,362)	*P*-value	Sample	Pheno-type	Family history of CAKUT	Additio-nal CAKUT	Extra-renal phenotype
2q11	del	96.96	97.24	0.28	2q11.2 deletion*	4	1	0	3.3×10^{-3}	KIM_1¶	RHD	N	N	Y
3q29	del	197.22	198.83	1.61	3q29 microdeletion	34	1	0	3.3×10^{-3}	KIM_2	MCDK	Y	Y	Y
15q13.3	del	28.52	28.76	0.24	15q13.3 microdeletion*	15	1	0	3.3×10^{-3}	KIM_1¶	RHD	Y	N	Y
16p13.3	dup	1.94	2.51	0.57	16p13.3 polycystic kidney disease*	42	1	0	3.3×10^{-3}	KIM_3	MCDK	Y	Y	N
16p13.11	dup	15.00	15.16	0.16	16p13.11 duplication	7	1	9	9.8×10^{-2}	KIM_4	URA	N	Y	N
16p12.2	del	21.75	22.32	0.57	16p12.1 distal deletion	17	1	16	5.4×10^{-2}	KIM_5	UPJO	N	N	N

CNV start and end positions are based on UCSC genome build hg18. The identified CNVs showed at least 70% overlap to a known genomic disorders, CNV-size >100 kb and frequency in controls <1:1,000. *P*-values for comparison of CNV frequency between cohort (n=80) and controls (n=23,362) are calculated using Fisher's exact test. *, These CNVs show ≥10% overlap with a known genomic disorder. ¶, Two rare known CNVs were identified in patient KIM_1. CAKUT, congenital anomalies of the kidney and urinary tract; CNV, copy-number variation; del, deletion; dup, duplication; MCDK, multicystic dysplastic kidney; UPJO, ureteropelvic junction obstruction; RHD, renal hypodysplasia and URA, unilateral renal agenesis.

TABLE 12.2. Novel rare genomic disorders identified in the KIMONO-GENE cohort.

Chr	CNV type	Start (Mb)	End (Mb)	Size (Mb)	N. of genes	KIMONO cohort (n=80)	Controls (n=23,362)	*P*-value	Sample	Phenotype	Family history of CAKUT	Additional CAKUT	Extra-renal pheno-type
1q44	del	246.41	246.71	0.30	12	1	5	9.8×10^{-3}	KIM_6	URA	N	N	Y
3p26	dup	1.13	1.64	0.50	2	1	5	6.6×10^{-3}	KIM_2*	MCDK	Y	Y	Y
4q25	del	113.14	113.54	0.40	6	1	0	3.3×10^{-3}	KIM_7	URA	N	N	Y
9q32	dup	115.88	116.21	0.33	9	1	0	3.3×10^{-3}	KIM_8	Complex CAKUT	N	Y	Y
10q21.1	del	57.04	57.32	0.28	1	1	5	2.0×10^{-2}	KIM_9	RHD	N	N	Y
13q21.32	del	66.10	66.41	0.32	1	1	1	3.3×10^{-3}	KIM_10	MCDK	Y	N	N
Xq12	del	65.68	65.93	0.26	1	1	3	1.3×10^{-2}	KIM_11	VUR	N	N	N

CNV start and end positions are based on UCSC genome build hg18. The identified novel rare CNVs have a size >250 kb and a frequency in controls <1:4,000. *P*-values for comparison of CNV frequency between cohort (n=80) and controls (n=23,362) are calculated using Fisher's exact test. *, Multiple CNVs were detected in subject KIM_2 (see Table 12.1). CAKUT, congenital anomalies of the kidney and urinary tract; CNV, copy-number variation; del, deletion; dup, duplication; MCDK, multicystic dysplastic kidney; RHD, renal hypodysplasia; URA, unilateral renal agenesis and VUR, vesicoureteral reflux.

are fundamental in helping explain a large fraction of the heritability for these traits, in formulating accurate diagnoses, in improving counseling, and in personalizing therapeutic strategies, CNVs offer major hurdles in their functional interpretation and in the identification of the underlying molecular defect that drives the phenotype(s) observed in our patients. When a CNV harbors multiple genes, different simplified scenarios can

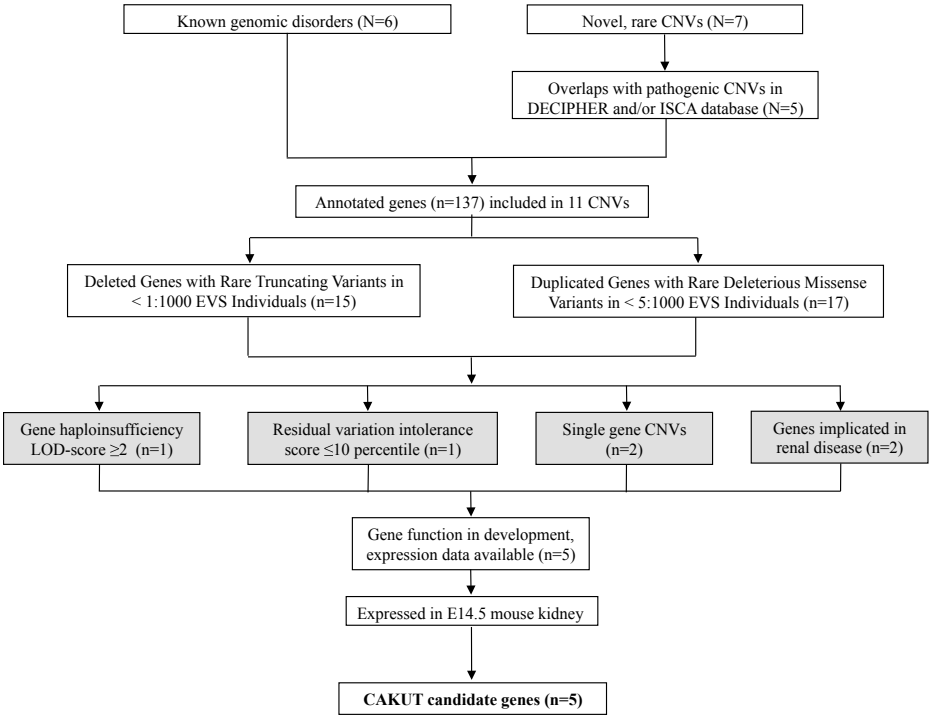


FIGURE 12.1. From CNVs to candidate genes for CAKUT.
All identified CNVs (N) were included in the analysis. For all novel, rare CNVs, deletions and duplications that showed significant overlap to pathogenic or uncertain pathogenic CNVs in public databases were included (see Supplementary Figure 12.1 and Supplementary Table 12.6). After annotation of gene content (n), genes that displayed rare truncating variants (deletions) and rare missense variants (duplications) in the Exome Variant Sever Database (http://evs.gs.washington.edu/EVS) were selected. We then assessed haploinsufficiency (HI-)LOD-scores and residual variation intolerance (RVI) scores for the prioritized genes (threshold values: HI LOD ≥2 and/or RVI-score <10th percentile) and included the prioritized genes within single gene CNVs as well as those genes that are implicated in renal disease. One gene met >1 threshold value for inclusion (*DLG1*). Gene expression profiles in the developing mouse kidney for all high-priority genes were evaluated by using GUDMAP (http://www.gudmap.org) and Genepaint (http://www.genepaint.org/) databases. Finally, we performed immunofluorescence studies in an E14.5 mouse kidney. By using this systematic bioinformatic approach, we prioritized 5 candidate genes for CAKUT. CAKUT, congenital anomalies of the kidney and urinary tract; CNV, copy-number variation and LOD, logarithm of the odds. Web-resources: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources Consortium (DECIPHER; http://decipher.sanger.ac.uk/); International Standards For Cytogenomic Arrays Consortium (ISCA; https://www.iscaconsortium.org/).

present: only one gene is the major driver for the phenotype, while the others have no or negligible effect; one gene is the main driver for the phenotype but the other genes act in an interactive manner to modify its penetrance and expressivity or multiple genes act as major drivers with reciprocal interaction effects.[325] The identification of genetic drivers for CNVs requires identification of independent mutations and functional proof of causation in animal models. When the single genetic driver model holds true, identification of independent coding point mutations and validation in genetically engineered mice is relatively straightforward, but as soon as the genetic architecture underlying the CNV becomes more complex, this approach is deemed to fail. Investigators leading this field have optimized assays to model loss- and gain-of-function mutations in a time- and cost-effective manner using zebrafish mutants,[332] which also allows testing for interaction. By using this approach, *KCTD13* has been identified as the major driver for the neuroanatomical phenotypes of loss or gain in copy-number on chromosome 16p11.2.[332] More recently, the same group was able to solve the molecular defects underlying the chromosome 8q24.3 duplication syndrome and to demonstrate interaction between *SCRIB* and *PUFA*.[333]

TABLE 12.3. Identified candidate genes for CAKUT by using an *in silico* systematic approach.

Gene	MIM	Corresponding CNV	HI-LOD score*	RVI-score**	RVI-percentile**	Genes included in CNV	Implicated in renal disease	Expression data in developing mouse kidney
DLG1	*601014	3q29 microdeletion	4.66	0.40	76.41	34	CAKUT (mice)	Yes
EDA2R	*300276	Xq12 deletion	−3.91	0.48	79.25	1	-	Yes
KIF12	*611278	9q32 duplication	NA	−0.13	43.98	9	Polycystic kidney disease (mice)	Yes
PCDH9	*603581	13q21.32 deletion	−1.09	−0.13	44.09	1	-	Yes
TRAF7	*606692	16p13.3 duplication	NA	−1.51	3.50	42	-	Yes

*, Based on Huang et al.[327] and **, based on Petrovski et al. [328]. Selected genes exhibited rare truncating variants (deletions) or rare missense variants (duplications) in the Exome Variant Server Database (http://evs.gs.washington.edu/EVS) and were subsequently dissected on the basis of at least one of the following criteria: individual gene HI-LOD-score ≥2, RVI-score ≤10 percentile, gene included in single gene CNV and/or gene implicated in renal disease (in mice or human). For all candidates, expression data in the developing mouse kidney was evaluated using GUDMAP (http://www.gudmap.org) and Genepaint (www.genepaint.org/). CAKUT, congenital anomalies of the kidney and urinary tract; CNV, copy-number variation; HI-LOD, haploinsufficiency logarithm of the odds; OMIM, Online Mendelian Inheritance in Man and RVI, residual variation intolerance score.

We recently reported on the potential for functional interpretation of genes underlying large pathogenic CNVs using extensive literature and database search to improve the diagnostic workup of a patient with a complex developmental phenotype comprising CAKUT, intellectual disability, limb defects, and other anomalies.[334] Here we propose an original pipeline to facilitate identification of high-priority genetic drivers for CNVs deriving from large datasets of genomic imbalances in order to narrow down the list of genes for follow-up resequencing studies and functional modeling in genetically engineered animal systems. These *in silico* analyses and expression studies in embryonic mouse kidney identified five potential candidate genes for CAKUT from a total of 151 transcriptional units underlying 13 rare CNVs.

Our findings strongly support *DLG1* as the main genetic driver of the renal phenotype for the 3q29 microdeletion syndrome and as a novel susceptibility gene for CAKUT, and provide the rationale for interpreting results from our analytical approach. First, the 3q29 microdeletion syndrome was not found in >23,000 population controls and it has been previously described in another individual with a horseshoe kidney.[335] Second, *DLG1* shows a very high haploinsufficiency LOD score (4.66), indicating that heterozygous deletions of this gene are unlikely to be tolerated.[327] Third, DLG1 is hypothesized to have a

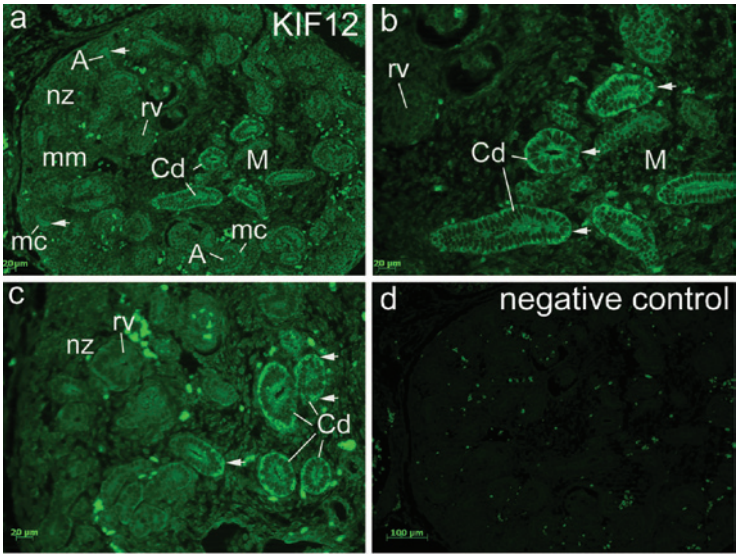


FIGURE 12.2. Expression of KIF12 in the developing renal mouse kidney.
Transversal section through lumbosacral part of mouse embryo (E14.5): within the nephrogenic zone (nz), Kif12 is weakly to mildly expressed (arrows) in the ampulla (A) and the developing nephron stages (metanephric cup – mc; renal vesicle – rv) and negative in the metanephric mesenchyme (mm). In the developing medulla (M), Kif12 is strongly expressed (arrows) in the epithelium of collecting ducts (Cd), and surrounding mesenchyme is negative; negative control (d). Immunostaining of Kif12, magnification ×10 (a, d), ×20 (b, c).

role in cellular polarity establishment, cell-cell adhesion and cellular proliferation[329] and *Dlg1* knock-out mice strikingly mirror the phenotypes observed in our patient, showing various abnormalities of the kidney and urinary tract, including renal hypoplasia, bilateral megaureter, duplex kidney and genital malformations,[329] and craniofacial malformations including cleft palate.[329,336] Consistent with these data, Dlg1 was expressed in the mouse developing urinary tract (Supplementary Figure 12.2). Altogether, these data suggest that our unbiased strategy can identify high-priority genetic drivers and CAKUT candidate genes from rare CNV data.

Consistently, we found a novel duplication of chromosome 9q32 that was absent in >23,000 population controls in a patient with a congenital megabladder, RHD and congenital VUR, which spans the *KIF12* locus. *KIF12* (Kinesin family member 12) plays an important role in intracellular transport and spindle formation during mitosis, and has been identified as a modifier gene for renal disease severity, defined by renal weight, length and volume, in autosomal recessive polycystic kidney disease in mice.[337] Moreover, the transcription of *Kif12* in mice is regulated by *Hnf1b*,[338] which is the most commonly implicated gene in isolated CAKUT.[17,18] We showed that *Kif12* is ubiquitously expressed in the developing nephrons and UB-derived structures on day E14.5 embryonic mouse kidneys (Figure 12.2). The gain of copy-number at this locus suggests increase gene dosage as a molecular mechanism, although loss-of-function cannot be excluded since the CNV proximal breakpoint is located only 16 kb from *KIF12* translation starting site.

Another potentially relevant structural variant was a deletion on the chromosome Xq12 locus in a child with isolated congenital VUR. This deletion disrupts *EDA2R* (Ectodysplasia A2 receptor), which has been proposed to cause hypohydrotic ectodermal dysplasia, a syndrome phenotypically characterized by hyperthermia, deficiency of sweat glands, hyperpigmentation around the eyes, sparse hair, eyebrows and eyelashes, and hypodontia with irregularly shaped teeth.[339] Although our patient did not show such phenotype, it is notable that a similar deletion on chromosome X is reported in the ISCA database in one patient with congenital hydronephrosis and intrauterine growth retardation.

The chromosome 16p13.3 duplication found in a girl with a prenatally involuted MCDK and an ureterocele was absent in >23,000 population controls and contains *PKD1*, *TRAF7* and *TSC2*. Loss-of-function mutations in *PKD1* and *TSC2* have both been implicated in renal disease (autosomal dominant polycystic kidney disease and tuberous sclerosis, respectively),[340-342] but our prioritization pipeline predicted *TRAF7* (Tumor Necrosis Factor Receptor Associated Family 7), which function is largely unknown and limited to the regulation of cell survival in meningiomas,[343] as the most likely genetic driver.

The remaining high-priority genetic driver identified here, *PCDH9*, is a member of the protocadherin family of genes and has predominantly been described within the central nervous system, where it is associated with a poor survival in glioma patients.[344]

In summary, we identified known or novel genomic disorders in 14% of patients with a solitary functioning kidney of congenital origin, confirming the importance of CNV analysis in improving diagnosis, risk stratification of developmental diseases that manifest later in life, and individualization of care. By using a novel prioritization strategy based on publicly available bioinformatic tools, coupled with expression profiling in the developing mouse urinary tract, we identified five potential genetic drivers underlying these CNVs, and propose *DLG1* and *KIF12* as high-priority novel candidate genes for CAKUT. Undeniably this approach presents several limitations and is designed to explore the most simplistic model in which the phenotype associated to a CNV is mostly driven by the effect of a single gene, but it provides a starting point for systematically annotate candidate genes from large datasets of CNVs in order to prioritize genes for follow-up resequencing and functional studies.

CONCISE METHODS

Additional Methods are reported in the Supplementary Materials.

Participants

The KIMONO-study, a large cohort study of over 400 children with a solitary functioning kidney has been previously described.[13] Individuals were enrolled from April 2012 until May 2013. The study protocol was approved by the institutional review boards of the VU University Medical Center and Columbia University Medical Center.

Genetic analyses

Sanger sequencing for HNF1B, PAX2, and DSTYK

Genomic DNA was purified from peripheral blood samples using standard methods. Specific primers were used to direct PCR at all exons and exon-intron boundaries of *HNF1B*, *PAX2*, and *DSTYK* as previously described (Supplementary Table 12.13).[16,18]

CNV analysis

Genome-wide genotyping was performed in all subjects using the Illumina Omni-Express platform (730,525 markers; Illumina Inc, San Diego, CA, USA). Genotype calls and quality controls were performed in GenomeStudio (v2011.1; Illumina Inc, San Diego, CA, USA) and PLINK.[345] The CNV calls were determined with generalized genotyping methods implemented in the PennCNV program.[346] Additional quality

control for CNVs were performed as previously described.[21] Annotation to the human reference genome and case-control association were performed using original Perl scripts generated in the lab.[21] We compared CNV data from cases to data derived from 23,362 anonymized adult and pediatric controls selected from eleven cohorts after stringent quality control. These controls include more than 13,000 individuals used in our previous CNV study on kidney malformations[21] plus additional ~10,000 individuals from dbGap studies [Verbitsky et al., *unpublished data*]. All controls had been genotyped on high-density Illumina platforms (Human-Hap-550, 610-Quad, 660W, 1M, 1M-Duo and Omni1) as part of case-control or longitudinal studies of complex traits. CNV frequencies were calculated on the basis of the entire control data set of 23,362 individuals. Based on our prior experimental validation on more than 100 CNVs, a confidence score ≥30 was used as threshold to define high-quality CNVs.[21] To minimize the different detectance rate for small structural variants between genotyping platforms with different coverage density, we restricted our analyses to CNVs ≥100 kb. We annotated CNVs with a frequency of ≤1:1,000 in controls with significant overlap (>10% in size) with known genomic disorders and subsequently used more strict frequency filters (<1:4,000) to identify potential novel genomic disorders. Finally, we annotated all rare intergenic CNVs that were absent in controls.

Systematic in silico analysis to identify genetic drivers for CAKUT-associated CNVs

To prioritize candidate susceptibility genes for CAKUT underlying rare CNVs, we developed an automated *in silico* approach that relies in publicly available bioinformatics tools (Figure 12.1 and Supplementary Methods).

Immunofluorescence staining

High-priority candidate genes for CAKUT from our *in silico* prioritization study were then tested for expression in the developing mouse kidney and urinary tract. Immunofluorescence staining on paraffin-embedded kidney tissues sections was performed on embryonic day E14.5 in C57BL/6 mice to investigate expression levels of all candidate gene products (except *PKD1* and *TSC2* as their role in human renal disease is well established).[341,342] The following rabbit polyclonal primary antibodies were used: anti-Dlg1 (55085-1-AP, also called SAP97), anti-Kif12 (12035-1-AP), anti-Traf7 (11780-1-AP; all three provided by Proteintech Group Inc., Chicago, IL, USA), anti-Eda2r (NBP1-76710), anti-Pcdh9 (NBP1-86073) and anti-Pcdh15 (NBP1-60065; all three provided by Novus Biologicals LLC, Littleton, CO, USA).

Statistics

Continuous variables are presented as mean (standard deviation; SD) or median [interquartile range; IQR] for variables with Gaussian and non-Gaussian distribution,

respectively. Qualitative variables are shown as counts (proportion). Difference in CNV frequency between cases and controls was tested using Fisher's Exact test and nominal *P*-values are reported.

Acknowledgments

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Web-resources

- 1000 genomes project: www.1000genomes.org/
- DECIPHER: http://decipher.sanger.ac.uk/
- Exome Variant Server: http://evs.gs.washington.edu/EVS
- International Standards For Cytogenomic Arrays Consortium (ISCA): https://www.iscaconsortium.org/
- Genepaint: www.genepaint.org/
- GenitoUrinary Development Molecular Anatomy Project (GUDMAP): http://www.gudmap.org
- National Center for Biotechnology Information - Single Nucleotide Polymorphism Database (dbSNP): http://www.ncbi.nlm.nih.gov/SNP/
- Polymorphisms Phenotyping, version 2 (Polyphen-2): http://genetics.bwh.harvard.edu/pph2
- UCSC Genome Bioinformatics: http://genome.ucsc.edu/index.htm

SUPPLEMENTARY MATERIALS

Supplementary methods

Sanger sequencing for HNF1B, PAX2, and DSTYK

Amplified PCR products were subjected to Sanger Sequencing and sequence traces analyzed and aligned to the reference genome using Sequencher 4.8 software (Gene codes corporation, Ann Arbor, MI, USA). Variants were annotated using dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), the 1000 Genome Project (http://www.1000genomes.org), and the Exome Variant Server database (EVS; http://evs.gs.washington.edu/EVS/). The probability of mutations causing damage was assessed by using the Polymorphism Phenotyping, version 2 software (Polyphen-2; http://genetics.bwh.harvard.edu/pph2/).

Systematic in silico analysis to identify genetic drivers for CAKUT-associated CNVs

First, we annotated all potentially pathogenic CNVs using the UCSC genome browser (http://genome.ucsc.edu/) to extract their entire gene content. We annotated all novel, rare genic CNVs, with structural variants of identical copy-number state and ≤10 Mb in size that were present in the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources Consortium (DECIPHER; http://decipher.sanger.ac.uk/) and in the International Standards for Cytogenomic Arrays Consortium (ISCA; https://www.iscaconsortium.org/). Rare CNVs were defined as potentially pathogenic when they showed significant overlap with pathogenic CNVs or CNVs with uncertain pathogenicity in DECIPHER or ISCA databases. We cross-annotated the genes within potentially pathogenic CNVs with the Exome Variant Server (EVS; http://evs.gs.washington.edu/EVS/) and excluded all deleted genes that carry truncating mutations in >1:1,000 individuals and all duplicated genes that carry deleterious missense variants in >5:1,000 individuals (Supplementary Table 12.7 and 12.8, respectively). Next, for all rare potentially pathogenic deletions, we calculated the per-CNV and per-gene HI-LOD scores as previously described.[327] Whole deletions and individual prioritized genes with a HI-LOD-score >2 are predicted to be subjected to HI and were considered to be potentially pathogenic. Similarly, genes underlying heterozygous deletions were interrogated for presence of loss-of-function truncating mutations (stopgain, splice, and frameshift) using the EVS Database. We then assessed genic intolerance to functional variation for all prioritized genes by using the RVI-score.[328] The RVI-score is based on the EVS database in order to develop a gene-level assessment that ranks genes in terms of likelihood for disease determination. In the validation of this score, developmental genes were significantly enriched in the lowest 25th percentile,[328] indicating a high intolerability for functional variants. To prioritize candidate genes for CAKUT, we used a more conservative threshold (10th percentile). Large rare single-gene CNVs were included in the prioritization pipeline, irrespective of HI-LOD or RVI-score, since they can point directly to the genetic defect. Finally, expression of all candidate genes in the developing mouse kidney was assessed by using the GUDMAP (http://www.gudmap.org) and Genepaint (www.genepaint.org/) databases, and known implication in renal disease in humans or mice was assessed using OMIM, MGI, PubMed and EMBASE searches.

Immunofluorescence staining

After deparaffinization and rehydration, slides were treated with hydrogen peroxide, washed in phosphate-buffered saline (PBS) and then cooked in sodium citrate buffer for 10 min at 95°C. After being cooled to room temperature, sections were blocked in a solution with goat serum and separately exposed to primary antibodies for 1 hour in a humidified chamber. Sections were subsequently incubated with a secondary antibody (Alexa fluor 488 donkey anti-rabbit, Invitrogen, OR, USA) for 1 hour at room temperature

and rinsed in PBS, mounted and coverslipped (Immuno-Mount, Shandon, Pittsburgh, PA, USA). Images were acquired using a motorized inverted microscope (Nikon TE2000, Nikon Instruments, Melville, NY, USA) and the images were captured using a HQ2 camera (Photometrics, Tucson, AZ, USA) and assembled using Adobe Photoshop CS2 Suite.

SUPPLEMENTARY TABLE 12.1. Patient characteristics of the KIMONO-GENE cohort.

	KIMONO-GENE (n=80)
Males (%)	54 (68)
Age at recruitment (years)	11.1 (5.6)
Ethnicity	80 (100)
Caucasian (%)	77 (96)
Asian (%)	1 (1)
Indian (%)	1 (1)
Hispanic (%)	1 (1)
All renal phenotypes	80 (100)
Renal agenesis, unilateral (%)	27 (34)
Renal hypodysplasia (%)	9 (11)
Multicystic dysplastic kidney (%)	30 (38)
Congenital vesicoureteral reflux (%)	10 (13)
Pelvi-ureteric junction obstruction (%)	2 (3)
Congenital megaureter (%)	2 (3)
Complex CAKUT (%)	37 (46)
Extra-renal anomalies (%)	26 (33)
Family history of CAKUT (%)	23 (29)

Data are presented as count (%) or mean (±standard deviation). CAKUT, congenital anomalies of the kidney and urinary tract.

SUPPLEMENTARY TABLE 12.2. Clinical characteristics of the KIMONO-GENE cohort.

	KIMONO-GENE (n=80)
Height SDS	−0.5 (1.5)
Weight SDS	0.0 (1.2)
BMI SDS	0.3 (1.2)
Systolic blood pressure SDS	0.3 (0.9)
Diastolic blood pressure SDS	−0.1 (1.1)
Serum creatinine (mg/dl)	0.61 [0.45-0.83]
eGFR (ml/min/1.73m^2)	97 [79-112]
Microalbuminuria (%)	15 (19)
Antihypertensive/antiproteinuric medication (%)	14 (18)
Renal length SDS	3.6 [1.4-4.8]

Data are presented as count (%), mean (±standard deviation) or median [interquartile range].
Body mass index (BMI) (kg/m^2) was calculated from weight and height. In addition, standard deviation scores (SDS) were calculated based on gender, age and ethnicity according to the Fifth Dutch Growth Study.[255] Blood pressure was measured with automated oscillometric devices with an appropriate cuff size. Hypertension was defined as a persistent presence of a systolic blood pressure and/or diastolic blood pressure ≥95th percentile corrected for age, gender and height.[243] Microalbuminuria was defined as a urinary albumin >30mg/24h – 300 mg/24h in timed collected urine samples or as a urinary albumin/creatinine ratio of >30 mg/g – 300 mg/g in a spot (morning) sample.[256] Serum creatinine (mg/dl) was measured enzymatically and eGFR was calculated using the Schwartz equation (eGFR= *k**height/serum creatinine in mg/dl), with a *k*-value of 41.3.[257] Renal length SDS was calculated based on data from two-kidney controls.[246,247] BMI, body mass index; eGFR, estimated glomerular filtration rate and SDS, standard deviation score.

SUPPLEMENTARY TABLE 12.3. Spectrum of single nucleotide variants identified in the KIMONO-GENE cohort.

Gene	Exon	Base change	AA change	Poly-phen2 score*	rsID	Frequency Caucausian (dbSNP)	Frequency Caucausian (EVS)	n (%)¶
DSTYK	1	c.190C>	p.L64F	0.027	rs78212113	0.017	0.022	1 (1)
DSTYK	3	c.655-30C>	-	-	rs76346293	0.0017	0.022	1 (1)
DSTYK	3	c.1294C>G	p.L432V	0.406	rs35845538	0.058	0.04	12 (15)
DSTYK	4	c.1384C>A	p.R462=	-	rs77626160	0.008	0.008	1 (1)
DSTYK	6	c.1809C>	p.S603=	-	Novel	-	-	1 (1)
DSTYK	7	c.1921T>C	p.C641R	0	rs3851294	0.117	0.07	11 (14)
DSTYK	8	c.2028C>	p.F676=	-	rs1062715	0.258	0.28	37 (46)
DSTYK	11	c.2353-59G>A	-	-	rs3851295	0.208	NA	28 (35)
DSTYK	12	c.*51T>A	-	-	rs113670	0.225	0.16	28 (35)
HNF1B	1	c.1-56	-	-	Novel	-	-	1 (2)
HNF1B	2	c.444G>A	p.S148=	-	rs147218489	0.00	0.00069	1 (2)
HNF1B	6	c.1339+27T>C	-	-	rs2107133	0.108	0.127	15 (25)
HNF1B	7	c.1340-100C>	-	-	rs9905004	0.050	-	5 (8)
HNF1B	8	c.1535-46T>G	-	-	rs2269842	0.449	0.283	19 (30)
HNF1B	8	c.1576+49C>	-	-	Novel	-	-	13 (21)
HNF1B	9	c.1576-22T>C	-	-	rs3110641	0.225	0.226	19 (32)
HNF1B	9	c.*99C>A	-	-	rs2229295	0.158	-	7 (18)
HNF1B	9	c.*100G>A	-	-	rs1800929	0.075	-	19 (30)
PAX2	1	c.43+10G>C	-	-	rs4472867	0.50	-	22 (28)
PAX2	1	c.43+43C>A	-	-	rs4405241	0.25	-	33 (41)
PAX2	7	c.792+9G>A	-	-	rs79552202	0.008	-	2 (3)
PAX2	8	c.798C>	p.N289=	-	rs1800897	0.025	0.137	7 (9)
PAX2	10	c.1022-43T>C	-	-	rs2270185	-	0.070	1 (1)
PAX2	11	c.*99C>	-	-	rs74729230	-	0.011	2 (3)

*, Scores for Polymorphism Phenotyping, version 2 (Polyphen-2) indicate the probability of the mutation causing damage. ¶ Sequencing data for *HNF1B* was available for 60 patients. The remaining 21 patients had been screened for *HNF1B* mutations in a prior study in which pathogenic mutations were absent. AA, amino acid; dbSNP, single nucleotide polymorphism database and EVS, exome variant server database. Web-resources: dbSNP (http://www.ncbi.nlm.nih.gov/SNP/); EVS (http://evs.gs.washington.edu/EVS/); Polymorphism Phenotyping (http://genetics.bwh.harvard.edu/pph2/).

SUPPLEMENTARY TABLE 12.4. CNV distribution in the KIMONO-GENE cohort.

	KIMONO-GENE (n=80)
Number of large CNVs	118
Average CNV rate per case	1.48
Median size (IQR) (bases)	174,844 [136,364-263,382]
No. of deletions (%)	50 (42)
No. of duplications (%)	68 (58)

The size of identified CNVs is ≥100 kb. CNV, copy-number variation and IQR, interquartile range.

SUPPLEMENTARY TABLE 12.5. Intergenic CNVs in the KIMONO-GENE cohort and absent in controls.

Chr	CNV type	Start (Mb)	End (Mb)	Size (Mb)	5′ gene	Distance to 5′ gene (kb)	3′ gene	Distance to 3′ gene (kb)	KIMONO cohort (n=80)	Sample	Pheno-type	Additional CAKUT	Extra-renal phenotype
2p13.3	del	71.77	71.81	0.03	PUM2	30.595	RHOB	28.394	1	KIM_13	MCDK	N	Y
2p24.1	del	20.44	20.48	0.04	DYSF	5.619	CYP26B1	402.68	1	KIM_4¶	URA	Y	N
3q11.2	del	100.25	100.29	0.04	DCBLD2	145.793	COL8A1	553.045	1	KIM_14	RHD	Y	N
7p21.3	del	8.82	8.98	0.16	NXPH1	56.363	NR_002790	663.577	1	KIM_15	MCDK	Y	N
10p14	del	6.96	6.97	0.01	AK094154	36.681	SFMBT2	269.603	1	KIM_7¶	URA	Y	Y
10q25.1	del	109.52	109.54	0.02	SORCS1	600.775	XPNPEP1	2075.479	1	KIM_16	URA	N	Y
11q22.1	del	103.00	103.03	0.03	DYNC2H1	145.831	PDGFD	254.461	1	KIM_18	URA	Y	N
13q14.2	dupl	46.61	46.94	0.31	HTR2A	243.48	SUCLA2	470.812	1	KIM_19	VUR	Y	Y
13q31.3	dupl	92.61	92.64	0.03	GPC5	296.806	GPC6	34.916	1	KIM_20	URA	Y	N
15q21.3	del	55.96	55.99	0.03	GRINL1A	163.898	ALDH1A2	42.595	1	KIM_21	URA	Y	N

CNV start and end positions are based on UCSC genome build hg18. Identified CNVs were intergenic and absent in controls. Distance to 5′ and 3′genes are indicated. ¶, Patients also had a known genomic disorder or a novel rare CNV (see Tables 12.1 and 12.2). CAKUT, congenital anomalies of the kidney and urinary tract; CNV, copy-number variation; del, deletion; dup, duplication; MCDK, multicystic dysplastic kidney; RHD, renal hypodysplasia; URA, unilateral renal agenesis and VUR, vesicoureteral reflux.

SUPPLEMENTARY TABLE 12.6. Phenotypes of patients in DECIPHER or ISCA-database overlapping novel rare CNVs in the KIMONO-GENE cohort.

Chr position	CNV-type	Size (Mb)	Database	Database ID	Phenotype information	Interpretation
chr1:246407223-246708850	Del	0.30	KIMONO	KIM_6	URA, Bilateral cryptorchidism, Dysmorphologies	Novel, rare
chr1:246368102-246547373	Del	0.18	ISCA	nssv583292	Developmental delay, Morphological phenotypes	Benign
chr1:246406596-246630481	Del	0.22	ISCA	nssv583883	Cryptorchidism, Hypotonia, Polyhydramnios, Pulmonary hyposplasia	Benign
chr1:246028892-246606419	Del	0.58	ISCA	nssv1495653	Developmental delay, Morphological phenotypes	Uncertain
chr1:237726884-247179291	Del	9.45	ISCA	nssv577257	Developmental delay, Morphological phenotypes	Pathogenic
chr1:237788353-247179291	Del	9.39	ISCA	nssv577258	Esophageal atresia	Pathogenic
chr1:237859791-247185415	del	9.33	ISCA	nssv577260	Developmental delay, microcephaly, polydactyly, syndactyly	Pathogenic
chr1:239277345-247185415	del	7.91	ISCA	nssv577261	Developmental delay, Morphological phenotypes	Pathogenic
chr1:239987174-247152131	del	7.16	ISCA	nssv577264	Agenesis of corpus callosum, Developmental delay, Seizures	Pathogenic
chr1:240306793-247179291	del	6.82	ISCA	nssv577265	Developmental delay, Morphological phenotypes	Pathogenic
chr1:242016554-247179291	del	5.16	ISCA	nssv577271	Developmental delay, Morphological phenotypes	Pathogenic
chr1:242016554-247179291	del	5.16	ISCA	nssv577272	Developmental delay, Morphological phenotypes	Pathogenic
chr1:242728796-247179432	del	4.45	ISCA	nssv577275	Developmental delay, Morphological phenotypes	Pathogenic
chr1:243814147-247179432	del	3.37	ISCA	nssv577278	Abnormal facial shape	Pathogenic
chr1:244616824-247179291	del	2.56	ISCA	nssv577279	Developmental delay, Morphological phenotypes	Pathogenic
chr1:245322357-247179291	del	1.86	ISCA	nssv577280	Developmental delay, Morphological phenotypes	Pathogenic
chr1:241935720-249197418	del	7.26	DECIPHER	249647	Cleft palate, Sloping forehead, Deeply set eye, Microcephaly, Delayed speech and language development, Delayed puberty, Intellectual disability, Muscular hypotonia, Seizures, Hypoplasia of the corpus callosum, Recurrent infections	NA
chr1:243170293-248658994	del	5.49	DECIPHER	249716	NA	NA

SUPPLEMENTARY TABLE 12.6 (Continued)

Chr position	CNV-type	Size (Mb)	Database	Database ID	Phenotype information	Interpretation
chr1:243181712-248675095	del	5.49	DECIPHER	249729	Low anterior hairline, Prominent nasal bridge, Seizures, Spasticity, Hypoplasia of the corpus callosum, Tetraplegia, Hypoplastic philtrum	NA
chr1:241469419-248865374	del	7.40	DECIPHER	250915	Microcephaly, Abnormality of the face, Low-set ears, Autism, Intellectual disability, Muscular hypotonia, Small feet, Proportionate short stature, Short palm, Midface retrusion	NA
chr1:241935720-249197418	del	7.26	DECIPHER	252695	NA	NA
chr1:243181712-248675095	del	5.49	DECIPHER	252731	NA	NA
chr1:245363407-249212668	del	3.85	DECIPHER	253276	NA	NA
chr1:246027111-249212668	del	3.19	DECIPHER	253729	Abnormality of the face, Intellectual disability, Abnormality of calvarial morphology	NA
chr1:241857038-249212668	del	7.36	DECIPHER	276524	Trigonocephaly, Progressive microcephaly, Cryptorchidism	NA
chr1:241935720-249197418	del	7.26	DECIPHER	249647	Cleft palate, Sloping forehead, Deeply set eye, Microcephaly, Delayed speech and language development, Delayed puberty, Intellectual disability, Muscular hypotonia, Seizures, Hypoplasia of the corpus callosum, Recurrent infections	NA
chr3:1134787-1636120	dup	0.50	KIMONO	KIM_2	MCDK, VUR, cleft palate	Novel, rare
chr3:1095841-1276800	dup	0.18	ISCA	nssv584563	Developmental delay, Morphological phenotypes	Benign
chr3:546267-1322293	dup	0.78	ISCA	nssv706166	Developmental delay, Morphological phenotypes	Uncertain
chr3:832141-1402460	dup	0.57	ISCA	nssv706173	Developmental delay, Morphological phenotypes	Benign
chr3:1578622-1681230	dup	0.10	ISCA	nssv706192	Developmental delay, Morphological phenotypes	Benign
chr3:1276800-2062270	dup	0.79	ISCA	nssv576334	Toe syndactyly	Uncertain
chr3:80526-1225893	dup	1.15	ISCA	nssv581086	Abnormal facial shape, developmental delay, hypotonia	Uncertain

SUPPLEMENTARY TABLE 12.6 (Continued)

Chr position	CNV-type	Size (Mb)	Database	Database ID	Phenotype information	Interpretation
chr3:641257-1358678	dup	0.72	ISCA	nssv581089	Developmental delay, morphological phenotypes	Uncertain
chr3:884817-1358819	dup	0.47	ISCA	nssv581090	Global developmental delay	Uncertain
chr3:1013431-1840696	dup	0.83	ISCA	nssv581091	Seizures	Uncertain
chr3:1172423-2001410	dup	0.83	ISCA	nssv581092	Global developmental delay	Uncertain
chr3:1467662-2771629	dup	1.30	ISCA	nssv581093	Autism spectrum disorder	Uncertain
chr3:1467662-2771629	dup	1.30	ISCA	nssv581094	Developmental delay, Morphological phenotypes	Uncertain
chr3:1108569-1897518	dup	0.79	ISCA	nssv582612	Abnormality of the heart	Uncertain
chr3:1514248-2237692	dup	0.72	ISCA	nssv583235	Global developmental delay	Uncertain
chr3:68949-5982771	dup	5.91	ISCA	nssv584388	Hypospadias, Inguinal hernia, Micrognathia	Pathogenic
chr3:1514248-2237692	dup	0.72	ISCA	nssv584461	Global developmental delay	Uncertain
chr3:885017-2317911	dup	1.43	ISCA	nssv706311	Developmental delay, Morphological phenotypes	Uncertain
chr3:207496-2062270	dup	1.85	ISCA	nssv706394	Developmental delay, Morphological phenotypes	Uncertain
chr3:943958-1322352	dup	0.38	ISCA	nssv1495222	Developmental delay, Morphological phenotypes	Uncertain
chr3:1490856-1877966	dup	0.39	ISCA	nssv1495223	Developmental delay, Morphological phenotypes	Uncertain
chr3:1013469-1715840	dup	0.70	ISCA	nssv1603934	Autism	Uncertain
chr3:832141-1389673	dup	0.56	ISCA	nssv1604921	Developmental delay, Morphological phenotypes	Uncertain
chr3:48602-3060004	dup	3.01	DECIPHER	248616	Narrow forehead, Long face, Intellectual disability, Hoarse voice, Joint laxity, Frontal bossing	NA
chr3:502222-1323399	dup	0.82	DECIPHER	249423	High palate, Microcephaly, Macrotia, Strabismus, Micrognathia, Thick eyebrow, Synophrys, Abnormality of the skin, Dysarthria, Intellectual disability, Seizures, Spasticity, Obesity, Abnormality of the voice, Pes planus, Hallux valgus, Intention tremor, Short nose, Abnormal dermatoglyphics	NA

SUPPLEMENTARY TABLE 12.6 (Continued)

Chr position	CNV-type	Size (Mb)	Database	Database ID	Phenotype information	Interpretation
chr3:501216-1857205	dup	1.36	DECIPHER	249581	NA	NA
chr3:501216-1766562	dup	1.27	DECIPHER	249687	Abnormality of the mouth, Abnormality of the gingiva, High palate, Bifid uvula, Cryptorchidism, Abnormality of the forehead, Prominent ears, Hypertelorism, Strabismus, Downslanted palpebral fissures, Abnormality of the nose, Wide nasal bridge, Micrognathia, Abnormality of the eyebrow, Spasticity, Apraxia, Downturned corners of mouth, 2-3 toe syndactyly	NA
chr3:501216-1857205	dup	1.36	DECIPHER	252662	NA	NA
chr3:501216-1766562	dup	1.27	DECIPHER	252769	NA	NA
chr3:180637-2660772	dup	2.48	DECIPHER	255761	Macrocephaly, Autism, Cognitive impairment	NA
chr3:35001-2233296	dup	2.21	DECIPHER	257903	Intellectual disability, Cerebellar vermis hypoplasia, Focal seizures with impairment of consciousness or awareness	NA
chr3:653211-1533505	dup	0.88	DECIPHER	260882	NA	NA
chr3:685234-1166340	dup	0.48	DECIPHER	262772	NA	NA
chr3:998906-1378848	dup	0.38	DECIPHER	266552	Microcephaly, Epicanthus, Hypertelorism, Intellectual disability, Sandal gap	NA
chr3:701645-2660772	dup	1.96	DECIPHER	269344	NA	NA
chr3:641287-1172653	dup	0.53	DECIPHER	272543	Intellectual disability, Feeding difficulties in infancy	NA
chr3:884817-2914900	dup	2.03	DECIPHER	277018	Generalized myoclonic seizures	NA
chr3:884817-2914900	dup	2.03	DECIPHER	277972	Generalized seizures, Seizures	NA
chr4:113141966-113543083	del	0.40	KIMONO	KIM_7	URA, ventriculoseptal defect	Novel, rare
chr4:112210390-117832483	del	5.62	ISCA	nssv584492	Developmental delay, Morphological phenotypes	Uncertain

SUPPLEMENTARY TABLE 12.6 (Continued)

Chr position	CNV-type	Size (Mb)	Database	Database ID	Phenotype information	Interpretation
chr4:111393648-115112024	del	3.72	DECIPHER	250341	Blue sclerae, Oligodontia, Intellectual disability	Pathogenic
chr4:110821159-113523378	del	2.70	DECIPHER	265977		Pathogenic
chr4:112297277-117431805	del	5.13	DECIPHER	247545	Failure to thrive, Neonatal hypotonia, Abnormal facial shape	Pathogenic
chr4:112213449-115723449	del	3.51	DECIPHER	278683	Aniridia, Congenital glaucoma	Pathogenic
chr9:115877566-116209120	dup	0.33	KIMONO	KIM_8	Megacystis, RHD	Novel, rare
chr9:117009156-117399361	dup	0.39	ISCA	nssv581370	Developmental delay, Morphological phenotypes	Uncertain
chr9:117009156-117399361	dup	0.39	ISCA	nssv581371	Developmental delay, Morphological phenotypes	Uncertain
chr9:117032675-117439804	dup	0.41	ISCA	nssv581372	Cafe-au-lait spot	Uncertain
chr9:117032675-117439804	dup	0.41	ISCA	nssv581373	Autism	Uncertain
chr9:117009169-117399454	dup	0.39	ISCA	nssv1602122	Developmental delay, Morphological phenotypes	Uncertain
chr9:117009169-117399454	dup	0.39	ISCA	nssv1604086	Developmental delay, Morphological phenotypes	Uncertain
chr10:57039123-57317587	del	0.28	KIMONO	KIM_9	RHD, cysts, duplex vagina	Novel, rare
chr10:55717005-58698309	del	2.98	ISCA	nssv577290	Seizures	Pathogenic
chr10:57579329-59272160	del	1.69	DECIPHER	249201	Intellectual disability, Seizures	NA
chr10:57129961-57627960	del	0.50	DECIPHER	253829	Short neck, Downslanted palpebral fissures, Conductive hearing impairment, Sensorineural hearing impairment, Prominent nasal bridge, Gynecomastia, Delayed puberty, Intellectual disability, Brachydactyly syndrome, Joint laxity, Highly arched eyebrow, Foot asymmetry, Fibular bowing	NA
chr10:56457421-59272301	del	2.81	DECIPHER	256920	Intellectual disability	NA

SUPPLEMENTARY TABLE 12.6 (Continued)

Chr position	CNV-type	Size (Mb)	Database	Database ID	Phenotype information	Interpretation
chr13:67201453-67516879	del	0.32	KIMONO	KIM_10	MCDK	Novel, rare
chr13:66225802-66246820	del	0.02	ISCA	nssv584686	Developmental delay, Morphological phenotypes	Benign
chr13:66205372-66304474	del	0.10	ISCA	nssv585031	Developmental delay, Morphological phenotypes	Uncertain
chr13:66113675-66297884	del	0.18	ISCA	nssv585054	Developmental delay, Morphological phenotypes	Uncertain
chr13:66024137-66595307	del	0.57	ISCA	nssv580358	Developmental delay, Morphological phenotypes	Uncertain
chr13:60856036-69261367	del	8.40	DECIPHER	271461	NA	NA
chr13:66138880-66537398	del	0.40	DECIPHER	277284	Alobar holoprosencephaly, 4-5 finger syndactyly	NA
chrX:65675658-65933752	del	0.26	KIMONO	KIM_11	VUR, RHD	Novel, rare
chrX:65654751-65941741	del	0.29	ISCA	nssv580671	Hydronephrosis, Intrauterine Growth Retardation	Uncertain
chrX:65544928-65883383	del	0.34	ISCA	nssv1604137	Developmental delay, Morphological phenotypes	Uncertain
chrX:65732215-65811740	del	0.08	DECIPHER	248305	NA	NA
chrX:65624549-65954565	del	0.33	DECIPHER	257738	NA	NA
chrX:65732215-65870024	del	0.13	DECIPHER	273518	Hyperactivity, Intellectual disability	NA

Chromosomal positions are based on UCSC build hg18. Mapped CNVs have identical copy-number as the novel rare CNVs (see Supplementary Figure 12.1). Phenotypes of patients as well as interpretation of the CNV (only available for ISCA) are additionally presented. The 1q44 deletion identified in individual KIM_6 shows significant overlap with two benign CNVs of similar size and was therefore not considered to be pathogenic. This was also observed in the 3p26 duplication identified in individual KIM_2 (overlap with 3 benign CNVs). All other CNVs were considered to include possible genetic drivers for the phenotype and were included in candidate gene prioritization. One patient from the ISCA database (nssv580671) with CAKUT (hydronephrosis) harbored a CNV that overlapped with the Xq12 deletion (KIM_11). MCDK, multicystic dysplastic kidney; NA, not available; RHD, renohypodysplasia; URA, unilateral renal agenesis and VUR, vesicoureteral reflux. Web-resources: USCS Genome Bioinformatics (http://genome.ucsc.edu/index.html); Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources Consortium (DECIPHER; http://decipher.sanger.ac.uk/); International Standards For Cytogenomic Arrays Consortium (ISCA; https://www.iscaconsortium.org/).

SUPPLEMENTARY TABLE 12.7. Genes within potentially pathogenic deletions with rare truncating mutations in the Exome Variant Database.

Gene	Chr	Start (Mb)	End (Mb)	Stop (%)	Splice (%)	Frameshift (%)
ZDHHC19	3	197.41	197.42	2/12,928 (0.02)	0	0
UBXN7	3	197.57	197.64	1/11,939 (0.008)	0	0
FBXO45	3	197.78	197.80	0	0	0
PAK2	3	197.99	198.04	0	0	0
SENP5	3	198.1	198.14	0	0	0
NCBP2	3	198.15	198.15	0	0	1/12,520 (0.008)
DLG1	3	198.26	198.51	0	1/12,518 (0.008)	4/37,556 (0.01)
BDH1	3	198.72	198.76	0	1/13,006 (0.008)	0
ARHGAP11B	15	28.71	28.72	0	0	5/11,443 (0.04)
UQCRC2	16	21.87	21.90	0	0	0
CDR2	16	22.27	22.29	0	0	0
C4orf32	4	113.29	113.33	0	0	1/12,518 (0.008)
TIFA	4	113.42	113.42	1/12,957 (0.008)	0	0
EDA2R	23	65.74	65.75	1/10,563 (0.009)	0	0
PCDH9	13	65.78	66.7	0	0	0

Gene start and end positions are based on UCSC build hg18. Data are presented as n/N (%). Frequencies of deleterious variants (stop, splice site or frame-shift mutations) in the Exome Variant Server Database (http://evs.gs.washington.edu/EVS) are presented for genes within pathogenic deletions. All variants identified are extremely rare in the healthy population (frequency <1:1,000 or <0.1%).

SUPPLEMENTARY TABLE 12.8. Genes within potentially pathogenic duplications with extremely rare deleterious missense variants in the Exome Variant Database.

Gene	Chr	Start (Mb)	End (Mb)	Missense (%)
NDUFB10	16	1.95	1.95	30/181,742 (0.02)
RPS2	16	1.95	1.95	4/51,888 (0.008)
NOXO1	16	1.97	1.97	20/180,642 (0.01)
SYNGR3	16	1.98	1.98	4/51,818 (0.008)
NTHL1	16	2.03	2.04	25/181,916 (0.01)
RAB26	16	2.14	2.14	24/153,208 (0.02)
TRAF7	16	2.15	2.17	21/194,264 (0.01)
RNPS1	16	2.24	2.25	14/129,940 (0.01)
C16orf59	16	2.45	2.45	22/161,074 (0.01)
TBC1D24	16	2.49	2.51	27/242,290 (0.01)
ATP6V0C	16	2.50	2.51	0 (0)
AMDHD2	16	2.51	2.52	33/216,420 (0.02)
NTAN1	16	15.04	15.06	19/90,958 (0.02)
AMBP	9	115.86	115.88	30/286,132 (0.01)
KIF12	9	115.89	115.90	20/234,108 (0.008)
ORM1	9	116.13	116.13	7/51,774 (0.001)
ORM2	9	116.13	116.14	22/142,932 (0.02)

Gene start and end positions are based on UCSC build hg18. Data are presented as n/N (%). Frequencies of missense variants in the Exome Variant Server Database (http://evs.gs.washington.edu/EVS) are presented for genes within pathogenic duplications. All variants identified are rare in the healthy population (frequency <5:1,000 or <0.5%).

SUPPLEMENTARY TABLE 12.9. Haploinsufficiency LOD scores for known deletions and novel rare deletions.

Chr	Start (Mb)	End (Mb)	No. of LOF genes	LOF genes with HI score	LOD score of deletion
2q11	96.96	97.24	6	1	−1.98
3q29	197.22	198.83	21	20	11.13
15q13.3	28.52	28.76	3	1	−2.04
16p12.2	21.75	22.32	12	6	1.45
4q25	113.14	113.54	4	4	−0.45
10q21.1	57.04	57.32	0	0	NA
13q21.32	66.10	66.41	0	0	NA
Xq12	65.66	65.93	1	1	−3.91

CNV start and end positions are based on UCSC build hg18. HI LOD-scores are based on Huang et al.[327] LOD score >2 is considered to be likely pathogenic. HI, haploinsufficiency; LOD, logarithm of the odds; LOF, loss of function and NA, LOD score not available.

SUPPLEMENTARY TABLE 12.10. Haploinsufficiency LOD- and Residual variation tolerance scores for prioritized genes within known genomic disorders and novel rare deletions.

Gene	Chr	Start (Mb)	End (Mb)	Haploinsufficiency LOD-scores			Residual variation intolerance score	
				No. of LOF genes	LOF genes with HI score	LOD score of deletion	RVI-Score	Percentile
ZDHHC19	3	197.41	197.42	1	1	−2.66551	NA	NA
UBNX7	3	197.57	197.64	1	1	−1.11102	NA	NA
FBXO45	3	197.78	197.80	1	1	−1.00849	−0.03107	51.03798
PAK2	3	197.99	198.04	1	1	1.42619	−0.33972	30.06605
SENP5	3	198.1	198.14	1	1	−2.19596	0.37668	75.5072
NCBP2	3	198.15	198.15	1	1	−1.07226	0.37668	75.5072
DLG1	3	198.26	198.51	1	1	4.65648	0.40054	76.40953
BDH1	3	198.72	198.76	1	1	−1.63484	0.42077	77.15853
ARHGAP11B	15	28.71	28.72	1	1	−2.03794	NA	NA
UQCRC2	16	21.87	21.90	1	1	−0.54415	0.21872	68.27082
CDR2L	16	22.27	22.29	1	1	−1.22054	−0.97907	8.75206
C4orf32	4	113.29	113.33	1	1	−2.21486	NA	NA
TIFA	4	113.42	113.42	1	1	−2.42919	−0.02925	51.40363
PCDH9	13	65.78	66.70	1	1	−0.76585	−0.12674	44.09059
EDA2R	X	65.74	65.75	1	1	−3.90861	0.48328	79.25218

CNV start and end positions are based on UCSC build hg18. HI LOD-scores are based on Huang et al.[327] A HI-LOD score >2 is considered to be likely pathogenic. RVI-scores are based on Petrovski et al.[328] The lower the RVI-score, the higher the likelihood that the gene is intolerant for functional variation (the percentile for the RVI-score is included). HI, haploinsufficiency; LOF, loss of function; NA, LOD score not available and RVI, residual variation intolerance score.

SUPPLEMENTARY TABLE 12.11. Residual variation tolerance scores for prioritized genes within known and novel rare duplications.

Gene	Chr	Start (Mb)	End (Mb)	Residual variation intolerance score	
				RVI-Score	Percentile
NDUFB10	16	1.95	1.95	NA	NA
RPS2	16	1.95	1.95	−0.56018	19.30880
NOXO1	16	1.97	1.97	0.24076	69.36778
SYNGR3	16	1.98	1.98	NA	NA
NTHL1	16	2.03	2.04	−0.58040	18.58929
RAB26	16	2.14	2.14	−0.27176	34.31824
TRAF7	16	2.15	2.17	−1.50825	3.50318
RNPS1	16	2.24	2.25	−0.18357	39.95046
C16orf59	16	2.45	2.45	0.46486	78.69191
TBC1D24	16	2.49	2.51	0.60078	82.82614
ATP6V0C	16	2.50	2.51	−0.14130	42.87568
AMDHD2	16	2.51	2.52	−0.19652	39.20736
NTAN1	16	15.04	15.06	0.08280	60.09082
AMBP	9	115.86	115.88	−0.48858	22.64685
KIF12	9	115.89	115.90	−0.13220	43.97853
ORM1	9	116.13	116.13	0.21690	68.12928
ORM2	9	116.13	116.14	0.79557	87.49115

Gene start and end positions are based on UCSC build hg18. RVI-scores are based on Petrovski et al.[328] The lower the RVI-score, the higher the likelihood that the gene is intolerant for functional variation (the percentile for the RVI-score is included). NA, RVI-score not available and RVI, residual variation intolerance score.

SUPPLEMENTARY TABLE 12.12. Overview of deleterious variants in prioritized loss-of-function candidate genes for CAKUT as identified in the Exome variant server database.

Gene	Exome variant server database		
	cDNA Change	GVS function	Frequency (%)
DLG1	c.1120-3_1120-2ins	splice-3	A1=1/R=12,517 (0.008)
	c.1007del1	frameshift	A1=1/R=12,519 (0.008)
	c.507_511del5	frameshift	A1=2/R=12,520 (0.016)
	c.75_76del2	frameshift	A1=1/R=12,520 (0.008)
EDA2R	c.757G>	stop-gained	A=1/C=10,563 (0.009)
PCDH9	None identified	NA	NA

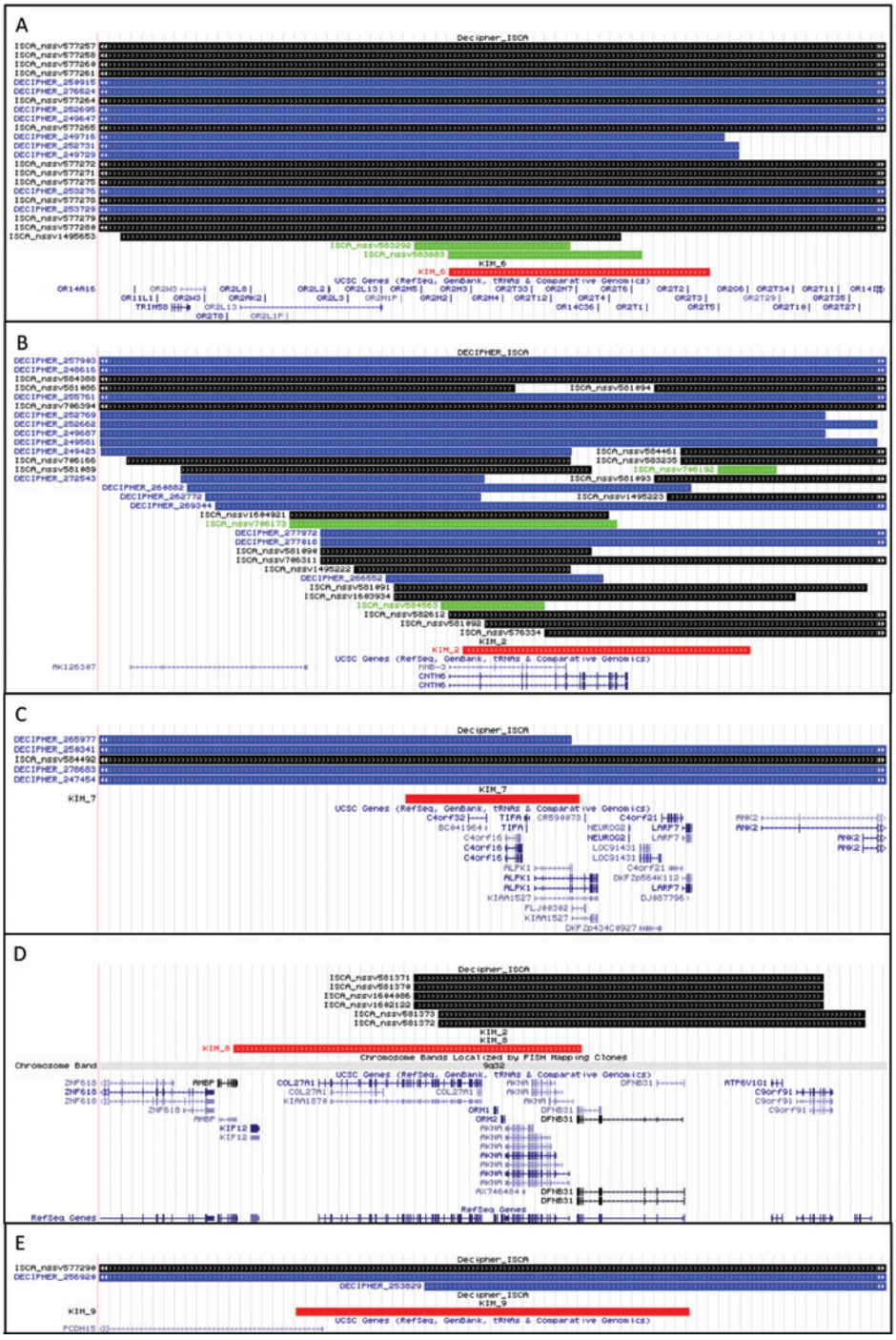
Frequency of deleterious variants (truncating, frameshift or splice site mutations) in the Exome Variant Server Database (http://evs.gs.washington.edu/EVS) for candidate genes identified within pathogenic deletions. All variants identified are rare in the healthy population (frequency <1:1,000), supporting the hypothesis that truncating mutations are susceptible to purifying selection.
GVS, genome variation server and NA, not applicable.

SUPPLEMENTARY TABLE 12.13. Exon specific primers for *HNF1B*, *PAX2* and *DSTYK*.

Gene	Exon	Direction	Sequence
HNF1B	1	Forward	TAACAGGTGTCTGGAGGCTG
	1	Reverse	GGCTTGGCGAGTGTGGTC
HNF1B	2	Forward	GGATGAGGTGTACCGTACAG
	2	Reverse	AGTGCTCACAAGGCCTTGTC
HNF1B	3	Forward	CTGCTGAGTGAAGGCTACAG
	3	Reverse	GAAGCTCTGATTTAGCCACAC
HNF1B	4	Forward	CCAAGACTGCTGTGATTGTG
	4	Reverse	AGATCCGTGGCAAGAACCAG
HNF1B	5	Forward	CCGAGTCATTGTTCCAGGAC
	5	Reverse	TTTGAGGCAGGCCTTGTGAG
HNF1B	6	Forward	CATCGTGTTGGAAACTGCTC
	6	Reverse	AGTTTGAAGGAGACCTACAG
HNF1B	7	Forward	ATCTCCTGTGTAACAGGCTC
	7	Reverse	ACTTCCGAGAAAGTTCAGAC
HNF1B	8	Forward	TCTACCTGAGGAGATGGGAG
	8	Reverse	GCTTGCCACAACCTCTGCAC
HNF1B	9	Forward	CTGCAGGAAGTGTGCCTCAG
	9	Reverse	TAAGCAGGGACCTCTCGCAG
PAX2	1	Forward	CCTCAAGTCCTGAAGTTGAG
	1	Reverse	GGCAGGTGATAGGGATCAG
PAX2	2	Forward	CCACCTTTCTTCTCAAGCTC
	2	Reverse	TTCAGCCACCATCTGAACAC
PAX2	3	Forward	AAGTCAGCTCAGCCACACTG
	3	Reverse	TGGACAAAGAGCAGAGACTG
PAX2	4	Forward	AATCGCTGAGGAACTTGGGA
	4	Reverse	TTCCTGCCTTTCTCTAGGTG
PAX2	5	Forward	CCTTATGTCCTCTGCTTCTC
	5	Reverse	GTCCAAGGACAAAGCATGTG
PAX2	6	Forward	CTGTGAGGGAATTGCAGCTC
	6	Reverse	TGAGGGCCAGAGGGAACA
PAX2	7	Forward	TCCTCAGCCAGATCTCTGAG
	7	Reverse	CAATGCTGGCTATGCATGTG
PAX2	8	Forward	CGGTTTCACCAAGTCAGGTC
	8	Reverse	TAGAAGCCTCGTTCTCTCTG
PAX2	9	Forward	GTACCCTGGTGTGAGTAGAG
	9	Reverse	CAGACCATTCAGCAGCTCAC
PAX2	10	Forward	ATGCCTCCTAGAACCGGAG
	10	Reverse	GTGCTGCACTAACAAGCCTG
PAX2	11	Forward	TTGTTCTCCTGTTTGTCCTC
	11	Reverse	GGTGATGTGAAGGGTTGCG
DSTYK	1	Forward	TTGTTTGCAACGGCAGTGAC
	1	Reverse	GTCCTCCGATTTGCCTCTC
DSTYK	2	Forward	TCATGGGATTTCTGCGTGAG

	2	Reverse	CTGCTGCTCAGAGTTCAGAC
DSTYK	3 (part 1)	Forward	GGAGGTGGATCAATGGCTG
	3 (part 1)	Reverse	TTCCACCAACATGCTCTGAG
DSTYK	3 (part 2)	Forward	ATAGACTCCTCAACCAGGAG
	3 (part 2)	Reverse	GTGGATGAAGTTACCCTGAG
DSTYK	4	Forward	ACGGTAGTTCCATGGTTCTG
	4	Reverse	GGCAGTGGTCATGTGACAG
DSTYK	5	Forward	ACCCTGAGTCTGGACTGTG
	5	Reverse	GGTCACCCTGCCTTAACAG
DSTYK	6	Forward	TTGTAGTGGTTCTTGTCTCTC
	6	Reverse	TCCCATCTGGATGAGGCTC
DSTYK	7	Forward	TGTGAGTATGCATCCAGGAG
	7	Reverse	CATTCCTCCTGCCTTATCTC
DSTYK	8	Forward	AGAGGCCATGCTGTAGCTC
	8	Reverse	CATGGCATGCAGGATTAGAG
DSTYK	9	Forward	AGACTCGTCTCAAACTCCTG
	9	Reverse	CCTGGGTTCAAGTGATTCTC
DSTYK	10	Forward	GTCTTTACTAGCAGCGTGAG
	10	Reverse	AGAGCATAATCCTATCCAGTG
DSTYK	11	Forward	GTGATTGTGGAAACGAGCTC
	11	Reverse	GTCAGCCTTTGAGCTGGTC
DSTYK	12	Forward	TGGGAGTGTGTCCTATAGTG
	12	Reverse	GGCAGGGTTGTTATTGAGAG
DSTYK	13	Forward	ATTGCCTGGGCTCAGTCTG
	13	Reverse	CATTCCTCCTGCCTTATCTC

Primers sequences used for amplification of the exons *HNF1B*, *PAX2* and *DSTYK*, which were subsequently subjected to Sanger sequencing.


A
Decipher_ISCA
UCSC Genes (RefSeq, GenBank, tRNAs & Comparative Genomics)
B
DECIPHER_ISCA
UCSC Genes (RefSeq, GenBank, tRNAs & Comparative Genomics)
C
Decipher_ISCA
KIM_7
UCSC Genes (RefSeq, GenBank, tRNAs & Comparative Genomics)
D
Decipher_ISCA
Chromosome Band
Chromosome Bands Localized by FISH Mapping Clones
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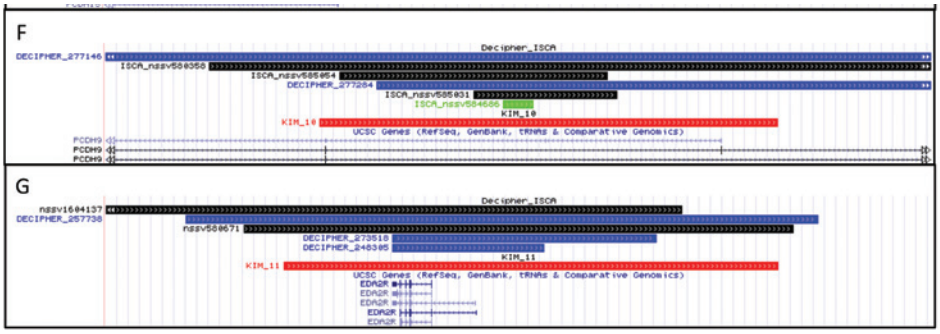
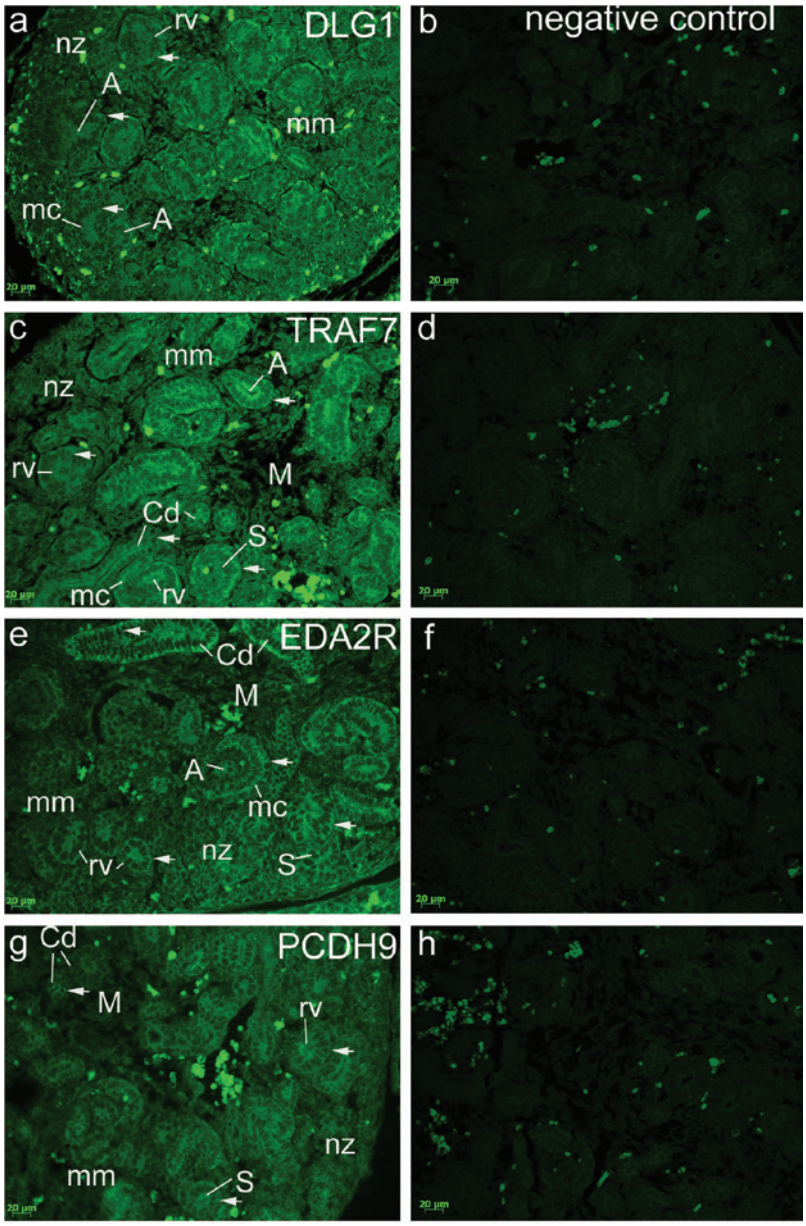


SUPPLEMENTARY FIGURE 12.1. Annotation of all identified novel rare CNVs to genomic disorders in ISCA and DECIPHER databases.

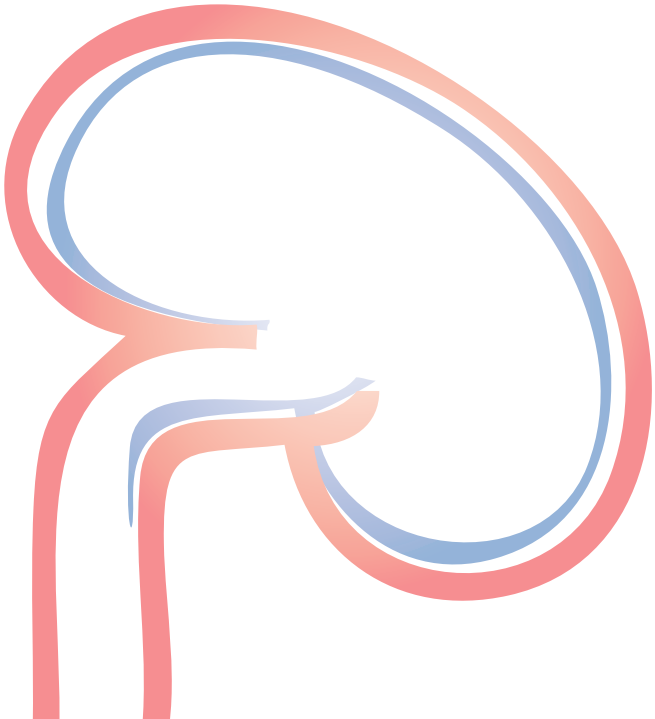
Alignment of all novel, rare CNVs (red) to CNVs with identical copy-number present DECIPHER or ISCA databases. (**a**) 1q44 deletion (301 kb), (**b**) 3p26 duplication (501 kb), (**c**) 4q25 (401kb), (**d**) 9q32 duplication (332 kb), (**e**) 10q21.1 deletion (278 kb), (**f**) 13q21.32 deletion (315 kb), and (**g**) Xq12 deletion (258 kb). Pathogenic CNVs and CNVs with uncertain pathogenicity are displayed in black (ISCA) and blue (DECIPHER). Benign CNVs are represented in green (ISCA). For every CNV, gene content within the CNV is displayed. Threshold size value for CNV alignment was defined as 10 Mb. Chromosomal positions are based on UCSC build hg18. Web-resources: UCSC Genome Bioinformatics (http://genome.ucsc.edu/index.html); Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources Consortium (DECIPHER; http://decipher.sanger.ac.uk/) and, International Standards For Cytogenomic Arrays Consortium (ISCA; https://www.iscaconsortium.org/).



SUPPLEMENTARY FIGURE 12.2. Expression of candidate genes in the developing renal mouse kidney. Transversal section through the lumbosacral part of an E14.5 mouse embryo. Within the nephrogenic zone (nz), Dlg1 (**a**), Traf7 (**c**), Eda2r (**e**), and Pcdh9 (**g**) are mildly expressed (arrows) in the ampulla (A) and developing nephron stages (metanephric cup – mc; renal vesicle – rv, comma-shape nephron –c, S-shape nephron – S) and negative in the metanephric mesenchyme (mm). In the developing medulla (M) only Eda2r is strongly expressed (arrow) in the epithelium of collecting ducts (Cd), and surrounding mesenchyme is negative; negative controls are presented in (**b**), (**d**), (**f**), and (**h**), respectively. Immunostaining of Dlg1, Traf7, Eda2r, and Pcdh9, magnification 20X.

Chapter 13

General discussion and future perspectives



INTRODUCTION

The objective of this thesis were to substantiate the risk for renal injury in children with a solitary functioning kidney and to design guidelines for the clinical monitoring of these patients. As many children within our cohort and reported in the literature have one or more signs of renal injury, our general findings indicate that a solitary functioning kidney in a child is a potentially harmful malformation. Moreover, our results should be appreciated in the perspective that the solitary functioning kidney of these children should last an entire lifespan, i.e. approximately 80 years.

In this chapter, we discuss our results by addressing the strengths and limitations of our studies and by defining recommendations for the clinical management of children with a solitary functioning kidney. Finally, we propose directions for future research in individuals with solitary functioning kidneys.

RENAL INJURY IN CHILDREN WITH A SOLITARY FUNCTIONING KIDNEY

Incidence of the solitary functioning kidney

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most commonly identified birth defects in humans (three to six per 1,000 live births[347]), and the predominant cause of end-stage renal disease in childhood.[1,81]

The solitary functioning kidney is an important clinical phenotype among the extensive spectrum of CAKUT. Results from this thesis indicate that the worldwide incidences of unilateral renal agenesis (URA) and multicystic dysplastic kidney (MCDK), both underlying the congenital type, are ~1:2,000 and ~1:4,300 births, respectively (**Chapters 3 and 4**). The incidence of an acquired solitary functioning kidney may be more variable, as it is dependent on the incidences of the several underlying phenotypes (e.g. CAKUT, renal malignancy, renal vascular stenosis, trauma), and remains to be established. Now that fetal ultrasonography is routinely performed in most developed countries, it is conceivable that the worldwide detection of both solitary functioning kidney types will further improve. Moreover, improvements in fetal imaging techniques (especially by magnetic resonance imaging [MRI][348]) will increasingly enable clinicians to predict the prognosis of solitary functioning kidney patients *in utero*. Fetal ultrasound informs about the underlying phenotype and insufficient renal hypertrophy, while additional CAKUT and/or extra-renal anomalies suggest syndromic forms of CAKUT. In addition, intrauterine growth retardation (IUGR) resulting in low birth weight is often suspected *in utero*. The timing of fetal ultrasound is also important for the detection of CAKUT, as a third trimester ultrasound has additional value to detect renal malformations.[116] Unfortunately, late pregnancy ultrasounds are not routinely performed in the Netherlands.

Nevertheless, fetal ultrasound can detect the majority of risk factors for renal injury studied in this thesis (**Chapter 7**), and ideally enables clinicians to estimate the clinical outcome of solitary functioning kidney patients in the earliest stages of life (Table 13.1).

Clinical outcome of the solitary functioning kidney from childhood

The rationale behind the KIMONO-study has been partly formed by the findings of a clinical study by Sanna-Cherchi and co-workers.[2] In their longitudinal study on the renal outcome of individuals with CAKUT, 20-50% of solitary functioning kidney patients developed end-stage renal disease at a mean age of 30 years, indicating that this CAKUT-phenotype has a very poor long-term prognosis. The renal outcome of solitary functioning kidney patients was further impaired when vesicoureteral reflux (VUR) was present (compared to a reference group of CAKUT phenotypes with the best clinical outcome: Hazard Ratio 7.50, 95% confidence interval 2.72 – 20.68).[2] Although a selected cohort of patients was described and the generalizability of the findings is therefore limited, this study has strongly contributed to the increasing concern about the outcome of patients with a solitary functioning kidney from childhood.[49]

In this thesis, we longitudinally studied the prognosis of children with a solitary functioning kidney (**Chapters 5-8**). We showed that ~1 in 3 individuals had signs of renal injury at a mean age of 9 years (**Chapters 5 and 7**), indicating that the aforementioned poor long-term prognosis is already discernable during childhood. Furthermore, generalized estimated equation models demonstrated that glomerular filtration rate (GFR) in these patients slowly declines from the beginning of puberty (**Chapter 5**). In addition, Kaplan-Meier survival analyses showed that 50% of subjects have at least one clinical sign of renal injury at a median age of 15 years of age (**Chapter 7**). Our findings are in accordance with Ardissino et al.,[349] who also emphasized the importance of puberty for disease progression in patients with chronic kidney disease (CKD). Hypothetically, the influence of gender and sex hormones on renal function may underlie this phenomenon,[268] but we were unable to detect these gender differences in adolescents with a solitary functioning kidney (**Chapter 8**). Disease progression during puberty may also be caused by the fact that there is insufficient renal reserve capacity (i.e. nephron number) to maintain normal GFR. Risk factors associated with renal dysfunction in these patients were increasing age, additional CAKUT and small renal size (Table 13.1). Furthermore, a history of urinary tract infections and a low birth weight (<2,500 g) were potentially associated with renal injury. Notably, these factors could all be associated with a reduced nephron number (Figure 13.1). Distal malformations of the urinary tract such as VUR or ureteropelvic junction obstruction can be accompanied by dysplasia of renal tissue, whereas a small renal size is a surrogate marker for renal hypo(dys)plasia. Recurrent upper urinary tract infections may cause renal scarring and nephron loss, while IUGR is associated with a lower nephron endowment in rats and humans.[250] Interestingly, the

developmental origin of health and disease (DOHaD) hypothesis implies that IUGR-patients have an increased risk to develop CKD in later life due to fetal programming.[54] One explanation for this is glomerular hyperfiltration in the lowered number of nephrons, suggesting a combination of the DOHaD and hyperfiltration hypotheses.

Gonzalez et al.[39] found that a body mass index (BMI) ≥25 kg/m^2 influences the progression of renal damage in adults with a solitary functioning kidney. Although we could not substantiate BMI as a risk factor in our pediatric cohort, obesity has been shown to induce glomerular hyperperfusion followed by an increased glomerular pressure and single nephron GFR, i.e. glomerular hyperfiltration.[350] The precise mechanism how obesity leads to glomerular hyperfiltration have remained elusive, but seems to be driven by impaired pressure natriuresis due to obesity-induced renin-angiotensin-aldosterone system activation.[350] The detrimental effects of obesity that become clinically evident in adults may not be detectable in childhood. Nevertheless, as the alarming trend of childhood obesity appears to increase,[351] adequate weight control in children with a solitary functioning kidney seems of cardinal importance.

Besides a reduced nephron number, the high incidence of renal hypodysplasia in the solitary functioning kidney could also underlie the impaired prognosis of this specific patient population. Dysplastic kidneys generally contain incompletely branched ducts surrounded by undifferentiated metaplastic stroma.[64] However, dysplasia may also be limited to a specific region of the kidney. For example, in the medulla, primitive tubules and cysts may have some excretory function,[64] whereas incomplete differentiation of the proximal tubular nephron segments causes renal tubular dysgenesis and often leads to anuria.[352] These perturbations in kidney development are likely to have systemic effects on blood pressure, glomerular filtration and tubular function. However, unraveling the exact pathogenic mechanisms requires further investigation.

Among the first studies on the impaired clinical outcome of individuals with a solitary functioning kidney, were two reports by Argueso and co-workers.[36,37] Using a retrospective design, they identified hypertension (47%), proteinuria (19%) and CKD (13%) in adult patients with URA.[37] Furthermore, 4% of patients died due to renal failure during follow-up. In a similar study on adults who acquired a solitary functioning kidney in childhood, Argueso et al. showed that 10% of individuals had hypertension, 27% had proteinuria and 30% had developed renal insufficiency during a median follow-up time of 24.7 years.[36] Important limitations of both studies are the induced selection and ascertainment bias, as not all patients had their blood pressure, urinary albumin excretion or GFR measured. Although the results from these studies can therefore not be generalized to all patients with a solitary functioning kidney, these are the scarce long-term follow-up data of individuals with a solitary functioning kidney from childhood known to date.

Other studies on short-term effects of renal mass reduction reported a milder outcome. In a cohort of 97 children with a solitary functioning kidney (44 congenital versus

53 acquired), Abou-Jaoudé and co-workers[35] found hypertension in 2% of patients, moderately increased albuminuria (previously indicated with the term 'microalbuminuria') in 18% of patients and a GFR <80 ml/min/1.73m^2 in 8% of patients. Interestingly, their

TABLE 13.1. Risk factors for renal injury as determined by the KIMONO-study.

Clinical parameter	Risk factor	Prenatal detection
Male gender	No	Yes
Increasing age	Yes	Not applicable
Acquired solitary functioning kidney	No	Not applicable
Ipsilateral CAKUT	Yes	Yes
Prenatal diagnosis	No	Yes
Birth weight <2,500 g	Possibly*	Yes
Body mass index	No	No
Urinary tract infections	Possibly*	No
Small renal size	Yes	Yes

*, Multivariable logistic regression demonstrated a trend (*P*-value ≥ 0.05 and < 0.10). CAKUT, congenital anomalies of the kidney and urinary tract.

data implied better functional adaptation in the congenital type, which is in line with our findings (**Chapter 7**). Several other studies excluded patients with abnormalities in the solitary functioning kidney, and found lower proportions of renal dysfunction.[34,40-42,70,130] All these studies display high variability in primary outcome measures studied, age at the time of study, methodology used and included phenotypes of solitary functioning kidneys. More importantly, many of these cohorts are assembled of solitary functioning kidney patients who had a clinical indication for an abdominal ultrasound (e.g. urinary tract infection, abdominal mass), thereby overrepresenting the more severe spectrum of this condition. This also holds true for the KIMONO-study (**Chapters 5 and 7**). In order to draw robust conclusions on the prognosis of all solitary functioning kidney patients, it is therefore important to perform longitudinal studies on large cohorts of children with a prenatally diagnosed solitary functioning kidney since their clinical outcome is less likely to be influenced by selection. Routine fetal ultrasound has been introduced in the Netherlands in 2007. Before this introduction, only mothers with an increased risk for a child with a congenital anomaly (e.g. due to a history of congenital defects or maternal illness) were screened. Hence, the follow-up time and number of patients with a prenatal diagnosis within the KIMONO cohort were relatively small. As a consequence, our analyses on the clinical outcome of children with a prenatal diagnosis remained inconclusive (**Chapter 7**).

Methodological considerations in renal injury studies

Although part of the children participating in the KIMONO-study cohort showed hypertension, albuminuria or CKD in childhood, the exact mechanisms underlying this renal dysfunction remain unknown. Glomerular hyperfiltration can only be assessed by measuring the total nephron number and single nephron GFR.[5] Normal nephron number in humans ranges from 200,000 to over 2.5 million nephrons per kidney,[6] and can only be determined *ex vivo* by using stereologic methods.[353] Despite the fact that renal size is often used as a marker for total nephron number, a recent meta-analysis has shown that this adult renal size only accounts for ~5% of the variation in nephron number.[354] Thus, although comparative studies in children are scarce,[354] we speculate that renal size is a very poor predictor for the estimation of nephron number.

Keller et al.[31] have provided indirect evidence for the glomerular hyperfiltration phenomenon in humans by showing that subjects with primary hypertension were endowed with half the nephron number of age-related controls. Furthermore, there was a 2.3-fold increase in glomerular volume of subjects with primary hypertension in comparison to age-related controls, suggesting glomerular hyperfiltration. Nevertheless, the question if renal injury in solitary functioning kidney patients is caused by glomerular hyperfiltration remains to be answered. Interestingly, a recent study has estimated nephron

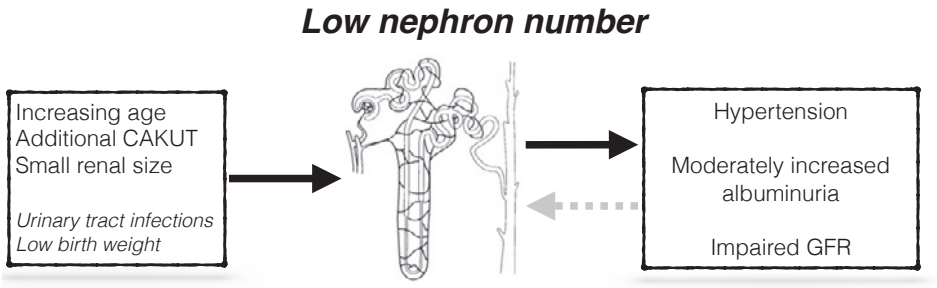


FIGURE 13.1. Risk factors for renal dysfunction in children with a solitary functioning kidney as identified in the KIMONO-study.
Independent risk factors are age, additional CAKUT and small renal size. Urinary tract infections and low birth weight (<2,500 g) demonstrated a trend for the development of renal injury. Hypertension, moderately increased albuminuria and impaired GFR may result in a vicious cycle leading to a further loss in nephron number. CAKUT, congenital anomalies of the kidney and urinary tract and GFR, glomerular filtration rate.

number in rat kidneys by using a robust MRI technique based on injection of cationic ferritin.[33] Further development of this technique might allow for the measuring nephron number *in vivo*, and the testing of the hyperfiltration hypothesis in solitary functioning kidney patients.

A surrogate marker for glomerular hyperfiltration is the filtration fraction (FF). FF can be calculated by simultaneously measuring gold standard GFR (e.g. inulin clearance) and renal plasma flow (by para-aminohippuric acid (PAH) clearance). Because these studies are cumbersome, costly and invasive, they are not routinely performed in daily pediatric practice. To assess the association between moderately increased albuminuria as an early prognostic marker for glomerular hyperfiltration, Cachat and co-workers[32] performed inulin and PAH clearance studies in 155 young adults with CAKUT (42 subjects had a solitary functioning kidney). They found a weak association between moderately increased albuminuria and an increased FF in the solitary functioning kidney group, which would limit the reproducibility of moderately increased albuminuria as a marker for hyperfiltration.

These findings were in contrast with the well-studied association in patients with diabetic nephropathy,[355] who may also be susceptible to glomerular hyperfiltration. By performing generalized estimation equation analyses, we showed that moderately increased albuminuria in solitary functioning kidney patients follows a similar trajectory as in diabetics (**Chapter 5**). By combining data from studies in diabetes patients and children with a solitary functioning kidney,[2,11,355] Schreuder discriminated different disease trajectories for patients susceptible to hyperfiltration (Figure 13.2).[5] These trajectories varied from normal renal function and normal range urinary albumin excretion to the development of hyperfiltration, moderately increased albuminuria and rapid decline in GFR.[5] Moderately increased albuminuria is considered to be a sign of increased "leakiness" of the glomerular endothelium, which can either be the consequence of a pressure-derived strain over the glomerular endothelium or caused by endothelial dysfunction.[356] The latter has been hypothesized as the most important explanation for the robust association between albuminuria and cardiovascular disease,[357] in which generalized endothelial dysfunction plays a significant role. However, albuminuria is not only a predictor for disease progression but also has direct damaging effects on the kidney. Animal studies have shown that overload of albumin and albumin-bound lipids are toxic to the proximal tubule and albumin-induced encroachment of the tubular glomerular junction may additionally lead to nephron loss.[358]

Importantly, similarities between solitary functioning kidney and diabetic nephropathy patients not only exist in disease progression but also in the treatment of disease. Blockage of the renin-angiotensin-aldosterone system by angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) has been shown to be effective in both groups.[52,359] The rationale for this treatment, amongst others, is to lower the intraglomerular pressure, thereby reducing the damaging effects of glomerular hyperfiltration on the kidney.

Given the overlap with diabetic nephropathy, moderately increased albuminuria thus seems an important prognostic factor in renal dysfunction. We showed that 19% of soli-

tary functioning kidney patients has moderately increased albuminuria at a mean age of 9 years (**Chapter 7**). In the KIMONO-study, urinary albumin excretion in 24h urine collections were used in most patients, which is the gold standard to determine moderately increased albuminuria in children. However, as this was not always feasible in children who are not fully toilet-trained, moderately increased albuminuria was determined in daytime random samples in the minority of patients. This method has been shown to be less precise than first morning voids.[56] As a consequence, benign orthostatic proteinuria might have inflated the incidence of moderately increased albuminuria in this limited number of patients.[56] We therefore conclude that more follow-up is needed to determine the prognostic value of albuminuria in solitary functioning kidney patients.

In this thesis, six common estimating equations for GFR (eGFR) in children with a solitary functioning kidney were validated (**Chapter 9**). Although these eGFRs have been designed for children with two kidneys, they are commonly used in solitary functioning kidney patients. As it is well-known that the accuracy of an eGFR depends on the test population,[283] it is mandatory to evaluate the performance of eGFR equations in solitary functioning kidney patients before used at bedside. By conducting Bland-Altman analyses,[290] we showed that a combined serum creatinine and cystatin C equation had the highest accuracy in this specific patient group, and, as a consequence, we recommend the use of this eGFR for children with a solitary functioning kidney. Our findings are in accordance with results from adults with CKD,[292] for who so-called "combined" equations provide the best estimation of GFR. However, as the availability of cystatin C is limited, the commonly used bedside eGFR by Schwartz et al.,[257] which includes a constant (*k*-value), the child's height and serum creatinine concentration, is a conceivable alternative.

Hypertension was determined using an automated oscillometric device in the renal injury studies (**Chapters 5-8**). Office blood pressure measurements cannot determine the influence of white coat hypertension, masked hypertension and isolated daytime and nocturnal hypertension.[55] Another important limitation of pediatric blood pressure data is the limited predictive value of childhood hypertension for adult hypertension, and the estimation of long-term cardiovascular outcome is modest at best.[360] In **Chapter 10**, we showed that ambulatory blood pressure monitoring (ABPM) is superior to office blood pressure measurement to identify hypertension in children with a solitary functioning kidney and should be implemented in the clinical management of these patients. However, ABPM in children has limited availability due to its high costs, may cause discomfort and could have imperfect reproducibility,[300] which has hampered the widespread use until now. Nevertheless, because hypertension is such an important prognostic marker for renal dysfunction in solitary functioning kidney patients, we feel that these limitations do not outweigh the advantages of accurate blood pressure

measurements. We therefore recommend future studies on renal injury to use ABPM in order to estimate the prevalance of hypertension in these patients.

Recommendations for the clinical management of children with a solitary functioning kidney

Despite the fact that CAKUT are the most important cause of end-stage renal disease in childhood, evidence-based guidelines for the clinical monitoring and management of patients with CAKUT, including solitary functioning kidney, have not yet been established. On the basis of current findings, however, increasing concerns about the prognosis of children with a solitary functioning kidney have risen.[3,45,49] Corbani et al.[45] proposed a careful approach to monitor these children from childhood, which includes evaluation of renal function and imaging studies for kidney size and VUR. If these studies identify additional CAKUT in the solitary functioning kidney, endoscopic or surgical intervention should be considered as well as long-term evaluations of blood pressure, urinary albumin excretion and serum creatinine. When additional CAKUT are absent, frequency intervals for follow-up depend on the presence of hypertension, moderately increased albuminuria and GFR. When any of these signs of renal injury are identified,

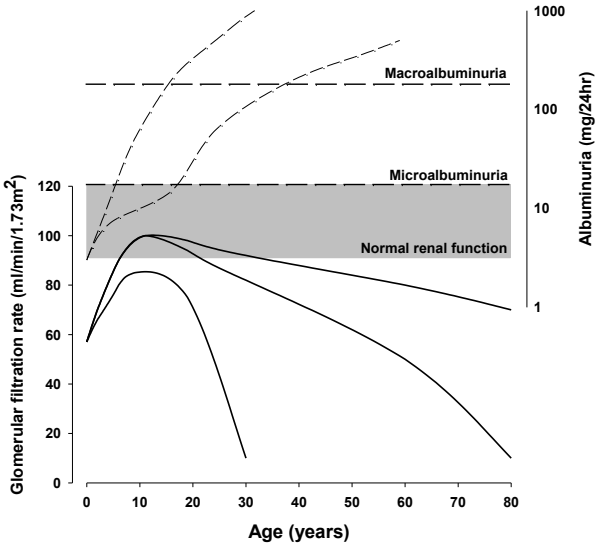


FIGURE 13.2. Different potential courses of albuminuria and glomerular filtration rate (GFR) based on the integration of data from previous studies (**Chapter 5**, [2,355]).
GFR (solid lines) may mature to normal two-kidney values (90-120 ml/min/1.73m^2) and gradually decrease during adulthood, with a gradual increase in albuminuria (dashed lines) into the moderately increased (30-300 mg/24h) or severely increased (>300 mg/24h) range. However, renal function may deteriorate much faster and lead to albuminuria in the first decade of life (**Chapter 5**) and end-stage renal disease in early adulthood[2] (Figure adapted from Schreuder[5]).

pharmacological interventions are proposed. If these patients remain asymptomatic until after puberty, the follow-up frequency can be prolonged to every 3-5 years. In The Netherlands, Van Den Hoek and co-workers[361] have provided guidelines for the clinical management of children with MCDK. The authors highlight that routine unilateral nephrectomy of MCDK to prevent the development of a renal malignancy or hypertension should be abandoned, and that the increased incidence of long-term effects such as moderately increased albuminuria and hypertension warrants regular clinical monitoring of MCKD patients throughout life.[361]

In this thesis, we propose opinion-based recommendations for the clinical follow-up of individuals with a solitary functioning kidney (**Chapter 1**; see also Table 13.2). Ideally, the diagnosis "solitary functioning kidney" is made by fetal ultrasonography, allowing for identification of additional anomalies during the fetal period and directly after birth, and appropriate counseling of family members. Additional imaging studies require renal ultrasound and renal isotope scans to identify potential ectopic functional renal tissue. Micturating cystourethrogram (MCUG) is the gold standard method to determine VUR in children, but is not always performed due to its invasive character. Despite the fact that MRI-based determination methods for VUR are in a developmental stage,[84] we feel that it is important to be informed about the presence of VUR in solitary functioning kidneys and MCUG should at least be performed in individuals with a high risk for VUR according to renal imaging studies (e.g. ureteral dilation or duplex collecting system).

To provide widely applicable clinical guidelines for follow-up, we have based our recommendations on two important risk factors, i.e. the presence of ipsilateral CAKUT and signs of renal injury. Differentiation in frequency intervals for follow-up should depend on these factors (Table 13.2). As a loss in GFR is generally preceded by hypertension and/or moderately increased albuminuria, blood pressure and urinary albumin excretion should be monitored at least once every year, even if CAKUT is absent. Serum creatinine measurement and estimation of GFR can be performed every 5 years. The follow-up intervals should be shortened when additional CAKUT is identified at diagnosis, as this predisposes to the development of renal injury. If signs of renal injury are detected, a more stringent approach is proposed with measurement of renal injury markers at least every 3 to 6 months. Ultrasound of the kidney and urinary tract should depend on the presence of additional CAKUT, such as a megaureter or pelviureteric junction obstruction, due to the fact that these complex CAKUT phenotypes may require surgical intervention to preserve renal function.

Ideally, GFR should be estimated using a combined serum creatinine-cystatin C equation (**Chapter 9**). However, as assays to determine cystatin C are not available in most clinical laboratories, the common eGFR-Schwartz is a reasonable alternative. If knowledge on the exact GFR is required, the single-injection inulin method is an accurate and minimally invasive gold standard GFR measurement in children.[269] Moreover,

this thesis demonstrates that ABPM is mandatory in the blood pressure management of individuals with a solitary functioning kidney (**Chapter 10**). When available, we recommend performing ABPM at least once a year to monitor blood pressure, as office measurements showed a tendency to miss hypertension in solitary functioning kidney patients.

Finally, although we are aware that our recommendations are limited by the lack of long-term follow-up, we feel that these frequencies allow for timely intervention to improve the overall clinical outcome of individuals with a solitary functioning kidney from childhood. In this regard, it is important for clinicians to not only focus on the renal prognosis but also on cardiovascular outcome, as moderately increased albuminuria and hypertension are both associated with cardiovascular disease such as coronary heart disease and stroke.[362] For example, animal studies have shown that sodium excretion in solitary functioning kidney is impaired, resulting in renin-angiotensin-aldosterone system activation and subsequently, arterial hypertension.[363] However, an impaired sodium excretion seems to predispose to coronary heart disease in humans, even before hypertension is detectable.[364] In our view, these findings further emphasize the importance to develop early therapeutic strategies in solitary functioning kidney patients to prevent CKD and its cardiovascular sequelae. In fact, ACEi and ARBs have shown to be effective to slow down disease progression in children with a solitary functioning kidney.[52]

Future directions of renal injury studies

It is complex to study the long-term prognosis of individuals with solitary functioning kidneys, as such studies require decades of follow-up. Continuation of the KIMONO-study cohort would allow us to design an individualized risk profile for renal dysfunction and tailor-made clinical guidelines for long-term follow-up of these patients. In order to do so, it is of critical importance to generate a well-defined cohort of unbiased, preferably prenatally diagnosed, patients. To establish such a cohort, more information on the national incidence of a solitary functioning kidney is required. Therefore, we are currently conducting a registration program in collaboration with the Dutch Pediatric Surveillance Unit (NSCK). This is a voluntary national electronic registry used by pediatricians to report various rare pediatric disorders. From January 2012 until January 2014, pediatricians in the Netherlands registered their newly diagnosed patients with a solitary functioning kidney. As there were approximately 176,000 children born in The Netherlands in 2012,[365] we expect an incidence of 80-90 children with a congenital solitary functioning kidney per year alone (**Chapter 3 and 4**). Furthermore, inclusion in the registration study also allows us to obtain long-term follow-up clinical data of these individuals. Preferably, this national study is a first step in establishing a large international working group on the clinical outcome of patients with CAKUT. Such initiatives, which

include pediatricians and adult nephrologists, are highly needed to develop evidence-based guidelines for the clinical management of individuals with CAKUT.

Longitudinal follow-up is mandatory to establish individualized risk factors for renal injury in solitary functioning kidney patients and to develop a clinically usable risk score. Ideally, this specific risk score is already used when the diagnosis of a solitary functioning kidney is made *in utero*, allowing clinicians to predict the prognosis of individual patients. However, the currently used markers for renal dysfunction are relatively insensitive and not specific. For example, a 50% loss of renal function is required for serum creatinine to come to medical attention.[366] Therefore, new surrogate markers for CKD have been tested in individuals with a solitary functioning kidney.[90,93] In this thesis, we used serum cystatin C based formulas to estimate GFR (**Chapter 9**). Other promising markers for renal dysfunction are neutrophil gelatinase-associated lipocalin (NGAL) and fibroblast growth factor-23 (FGF23).[267,366] Recently, FGF23 has been identified as the principal hormone in the renal handling of phosphate homeostasis.[367] Interestingly, patients with CKD have 100-10,000 fold increases in serum FGF23 levels compared to healthy controls.[368] This increase might reflect the direct response to the diminished renal clearance of phosphate due to a loss of functioning nephrons, as FGF23 down-regulates the formation of phosphate transporters in the proximal tubule of the kidney and reduces systemic levels of 1,25-dihydroxyvitamin D.[367] This rise in FGF23 is much earlier and more abrupt than serum creatinine, suggesting that FGF23 is a more sensitive marker for CKD. In addition, Bacchetta and co-workers[267] validated the inverse association between FGF23 and GFR in 227 children with CKD (including 20 solitary functioning kidney patients). However, several issues exist in the implementation of FGF23 in standard clinical care. First, although the pathophysiological significance of FGF23 is

TABLE 13.2. Opinion-based recommendation for clinical follow-up intervals of children with a solitary functioning kidney.

		Blood pressure	Urinalysis	Serum creatinine / GFR	Renal ultrasound
No renal injury	*CAKUT -*	One time per year	One time per year	Every 5 years	Every 5 years*
	CAKUT +	Two times per year	Two times per year	Every 5 years	As indicated
Renal injury	*GFR <60 ml/min/1.73m^2 or medication for proteinuria/ hypertension*	Two to four times per year	Two to four times per year	Two to four times per year	As indicated

The presented follow-up intervals are based on risk assessment at time of diagnosis. *, Last ultrasound to be performed at 15-16 years of age.

becoming increasingly clear, the precise roles and cellular mechanisms of FGF23 and its cofactor Klotho in CKD remain to be elucidated.[369] Secondly, the measurement of FGF23 has not been standardized. As there are multiple FGF23 assays, all with different units and consequently, different reference values, there is a strong need for uniformity in the determination of FGF23 concentrations. Reference values are indispensable to use FGF23 as a renal marker for pediatric CKD. However, there is a strong heterogeneity among pediatric FGF23 studies, resulting in absence of pediatric standardized values. Therefore, our recent attempt to develop pediatric reference values by performing a systematic review and meta-analysis of the literature remained unsuccessful [Abraham et al., *unpublished data*]. Finally, the determination of FGF23 is more expensive and still confined to specialized clinical laboratories, limiting its availability for current clinical care.

The emerging role of FGF23 emphasizes the importance of the tubulointerstitium in the onset and progression of renal disease.[363,370] Glomerular hyperperfusion can lead to direct damage of the tubulointerstitium resulting in renal fibrosis due to compensatory immunological responses and loss of renal concentration capacity, acid-base homeostasis and electrolyte handling.[363] The studies described in this thesis have mostly focused on the direct (glomerular) effect of a reduced renal mass. Challenges remain to study the tubular function of individuals with a solitary functioning kidney, for example by measuring of NGAL, kidney injury molecule-1 (KIM-1) and beta2-microglobulin as markers of tubulointerstitial damage. Notably, renal tubular dysfunction has recently been identified in type 1 and type 2 diabetic patients with glomerular hyperfiltration.[371,372] Once the molecular targets in the development of tubulointerstitial damage are identified, the next step includes the development of therapeutic strategies to disrupt the ongoing detrimental effects of hyperfiltration on the tubulointerstitium.

Finally, the development of new therapeutic strategies is mandatory to improve the outcome of individuals with solitary functioning kidneys. An important potential target to do so is enhancing nephrogenesis *in utero*. Data from animal studies have suggested a compensatory rise in nephron number in individuals with a congenital solitary functioning kidney (**Chapter 1**). However, this rise only reaches approximately two-thirds of the total nephron number identified of two kidney controls.[363] If glomerular hyperfiltration in solitary functioning kidney patients does take place, establishing a 100% increase in single kidney nephron number would prevent the occurrence of its detrimental effect in these children. This would not only affect the outcome of the congenital type, but may also be important to children with an acquired solitary functioning kidney because many of the underlying phenotypes leading to nephrectomy in childhood are in fact congenital (e.g. pelviureteric junction obstruction, megaureter or high grade VUR. An even broader perspective on the benefits of enhancing nephrogenesis would be children with IUGR and prematurely born neonates (i.e. <34 weeks of gestation), as these patients are known

to have a reduced nephron number and an increased risk for CKD.[5,54,373] Now that our understanding of the complex interactions involved in nephron formation is improving, such therapeutic strategies will hopefully be developed over the coming decades.

Furthermore, preclinical (animal) models allow us to develop therapeutic strategies in children with renal mass reduction. To reduce the risk for CKD in individuals with solitary functioning kidney, it is important for clinicians to be informed about the timing of therapy (pre- versus post-pubertal) and to evaluate the effectiveness of various antihypertensive/antiproteinuric agents (e.g. combination of ACEi/ARBs with calcium antagonists, beta blockers or new agents such as Sodium-Glucose Linked Transport 2 (SGLT2) inhibitors[374]). For example, it has been shown that early treatment with ACEi in the spontaneously hypertensive rat prevents the development of hypertension in these animals, whereas late treatment did not result in blood pressure normalization.[375] Although the exact mechanisms have not been elucidated, one can speculate that early ACEi leads to a change in the set point of the renin-angiotensin-aldosterone system activation thereby normalizing blood pressure in these rats. Hypothetically, early treatment with ACEi in a toddler with a solitary functioning kidney may thus have lifelong benefits. Experimental studies on renal mass reduction should ideally be conducted in an fetal nephrectomy ovine model,[363] as renal development in sheep is closely related to nephrogenesis in humans.

The next step to lower the risk for CKD and end-stage renal disease in individuals with solitary functioning kidneys will include randomized controlled trials in adult *and* pediatric patients with renal mass reduction. Until now, only one randomized trial has been performed in children with renal hypodysplasia,[52] which showed that stringent blood pressure control and a decrease in urinary albumin excretion with ACEi delays the natural disease progression. This number is in strong contrast with the volume of studies investigating therapeutic strategies of diabetic patients with glomerular hyperfiltration.[376] Nevertheless, randomized controlled trials in a large cohort of patients are indispensable to optimize the clinical management of children with a solitary functioning kidney, and, using an even broader perspective, other individuals with severe phenotypes of CAKUT and low nephron endowment, such as posterior urethral valves, bilateral pelviureteric junction obstruction and bilateral renal hypodysplasia. Performing the above-mentioned studies therefore will not only improve the prognosis of individuals with a solitary functioning kidney, but also provide a rationale for future studies in children with severe renal malformations.

GENETICS OF CAKUT AND SOLITARY FUNCTIONING KIDNEY

Complex genetic make-up of CAKUT

Human renal development begins during the fifth gestational week when growth factors initiate growth of the ureteric bud (UB) from the mesonephric duct towards the metanephric mesenchyme (MM).[14] Failure of adequate interaction between the UB and the MM perturbs normal renal development. CAKUT can often be aggregated into complex phenotypes with several simultaneously affected segments of the urinary tract,[7] which hampers its classification and thus, diagnosis.[12] Underlying this high phenotypic variability is the complex genetic architecture of CAKUT, characterized by a variable mode of inheritance (X-linked, autosomal dominant and recessive inheritance have been described[12,16,308]), familial aggregation[114] and the fact that the same genetic mutation can have pleiotropic effects.[12]

In **Chapters 1 and 11** of this thesis, we describe the pivotal role of genetic factors in CAKUT and solitary functioning kidney. Gene discovery for this trait has been relatively slow due to high genetic heterogeneity, incomplete penetrance of disease and the small pedigree sizes studied (**Chapter 11**). Most of the genes implicated in CAKUT have been identified in syndromic forms of renal maldevelopment, and are often characterized by their extra-renal manifestations (**Chapter 1**). Nevertheless, several genes have been found causative in isolated forms of solitary functioning kidney. In one of the first large mutation screens of 100 patients with renal hypodysplasia, Weber and co-workers[17] reported pathogenic mutations in *PAX2* and *HNF1B* in 15% of patients. Many of these patients (23%) had extra-renal malformations associated with renal hypodysplasia. In a similar study describing renal hypodysplasia patients participating in the Chronic Kidney Disease in Children Cohort (CKiD) study, Thomas et al.[18] found disease-causing mutations in *PAX2* and *HNF1B* in 10% of patients. A substantial proportion (30%) of subjects had a positive family history for renal disease. Recent implementation of whole-exome sequencing techniques for gene discovery in CAKUT have identified mutations in *DSTYK*, encoding for a dual specificity serine/threonine and tyrosine kinase, in 2.3% of patients with renal malformations.[16] Aggregation of all pathogenic mutations therefore allows to establish a molecular diagnosis in only ~20% of cases.

Recently, structural variants were identified as a common underlying cause of CAKUT.[21] Structural genic variants can be identified in ~8% of the healthy population[377] and mainly constitute insertions, deletions and duplications of the genome. In the last decade, it has been increasingly recognized that these variations in copy-number (CNVs) are the cause of genomic disorders, especially in individuals with neurodevelopmental delay and neurocognitive defects such as autism and schizophrenia.[320] Although the exact pathogenic mechanism seems complex and remains to be elucidated, these variants are likely to compromise the function of several genes at once, implicating multiple ge-

netic drivers for disease.[325] By using high-density genotyping arrays in 522 patients with renal hypodysplasia, Sanna-Cherchi et al.[21] showed that pathogenic CNVs are enriched among affected individuals when compared to almost 14,000 healthy controls. This allowed them to establish a molecular diagnosis in 17% of renal hypodysplasia patients.

In this thesis, we performed a similar approach to validate these previous results in the KIMONO-cohort (**Chapter 12**). We found known and novel pathogenic CNVs in 14% of children with a solitary functioning kidney. A substantial proportion (50%) of patients harboring these genomic disorders had extra-renal anomalies. As rare point mutations in the three most common disease-causing genes (i.e. *HNF1B*, *PAX2* and *DSTYK*) were excluded in our cohort, these findings emphasize the major role of gene dosage imbalances in the pathogenicity of CAKUT and strongly advocate the search for genomic disorders in individuals with solitary functioning kidneys. Furthermore, in order to define novel candidate genes that may predispose to CAKUT, we performed a systematic *in silico* analysis by using available bioinformatics tools (**Chapter 12**). Five novel candidate genes for CAKUT were identified (i.e. *DLG1*, *EDA2R*, *KIF12*, *PCDH9*, and *TRAF7*). Expression of all genes was evaluated in the developing kidney of an embryonic mouse and confirmed by immunofluorescence testing. Among the identified candidate genes were *DLG1* and *KIF12*, which have been previously associated with renal developmental disorders in animals and humans.[329,337] These findings implicate that our *in silico* analysis includes a robust approach to define candidate genes for CAKUT.

Methodological considerations in genetic studies

The strengths of the KIMONO-GENE study are the well-defined clinical cohort, the uniform high-resolution genotyping platform used, and the fact that data from over 23,000 healthy adults and pediatric controls were implemented to identify extremely rare pathogenic genomic disorders. The findings of our study provide a first step in the validation of pathogenic variants in congenital renal defects. However, to unravel the complex genetic make-up of CAKUT, functional studies using animal models are essential to evaluate the pathogenicity of the identified candidate genes and large cohorts of CAKUT patients are required to identify rare pathogenic variants implicated in human disease.

In our study, stringent computational quality control was used to identify CNVs that were rare or absent in healthy controls and had a high confidence score. In addition, we considered exonic CNVs >100 kb in size as a threshold value for pathogenicity. However, pathogenic CNVs can be much smaller in size (up to 1 kb).[325]

Furthermore, as our understanding of the genetics of renal diseases is increasing, intronic variants may become more important. These non-coding regions of the genome contain many regulatory elements required for normal cell function. One example of the importance of introns in renal development is MicroRNA (miR)-17~92, which is a small

noncoding RNA that regulates gene expression in nephron progenitor cells. Conditional deletion of miR-17~92 was found to cause renal hypodysplasia in mice, leading to albuminuria and glomerulosclerosis within 3 months after birth.[378]

Another important limitation of our study is the fact that we did not have DNA from family members available to test the inheritance of CNVs. As there is a strong correlation between *de novo* rate and CNV size,[320] trio-based genotyping studies (i.e. genetic studies of the index patient, father and mother) are required to further determine the pathogenicity of CNVs.

Finally, the developing kidney is vulnerable to environmental influences such as nephrotoxic drugs (especially aminoglycosides, prostaglandin synthetase inhibitors and ACEi)[22] and prenatal exposure to alcohol.[379] Exposure to these teratogens has been associated with CAKUT and should therefore be reduced to a minimum during gestation, particularly in children with a solitary functioning kidney. Our study did not take these environmental factors into account (**Chapter 12**). Neonates born before 32 weeks of gestation are particularly susceptible to environmental influences that perturb normal renal development (**Chapter 1**), as 62% of them are treated with aminoglycosides in the Netherlands.[22] Future studies on the combined role of genetic and environmental factors in the development of CAKUT are therefore highly needed to study the natural history of renal malformations and design targeted therapeutic strategies.

Future perspectives on genetic studies in solitary functioning kidney

Major challenges lie ahead for gene discovery studies in solitary functioning kidney and CAKUT. Given their complex genetic make-up, CAKUT have been defined as "the disease of hundreds of genes". However, as children with complex CAKUT increasingly reach the reproductive age due to the tremendous improvements in prenatal, neonatal and pediatric care, it is likely that the incidence of CAKUT patients in the population of nephrology patients will gradually increase. As a consequence, our genetic understanding must increase in order to establish a correct diagnosis and provide appropriate genetic counseling for such patients and their family members.

Recent improvements in genetic techniques and computational analyses enable us to rapidly identify candidate genes for CAKUT. However, functional studies and large cohorts of CAKUT patients remain indispensable in order to dissect pathogenic mutations from variants that are not causing disease. As the currently known pathogenic variants in autosomal dominant CAKUT are identified in the minority of patients, large international collaborations between pediatric renal centers and genetic laboratories are mandatory for gene discovery studies. Such efforts are being made more and more often. For example, three recent studies were successful using whole-exome sequencing techniques in large cohort of CAKUT patients (>300 cases) to replicate mutations in candidate genes for CAKUT.[16,309,380] It is likely that such studies will become more abundant

in the upcoming years, especially with the availability of targeted sequencing methods, which encompasses a low-cost technique to evaluate the most commonly implicated CAKUT genes.[381]

Regarding the identification of CNVs, it is likely that whole-exome sequencing (and eventually, whole-genome sequencing) will gradually replace high-density platforms as the preferred method to investigate structural genomic variants.[319] Although next-generation sequencing will allow for higher density analyses, several technological and financial challenges remain to use this technique for structural variants identification.[319] Before the next available technology should be introduced, large collaborations between investigators are warranted to cross-validate and replicate findings coming from exome sequencing or CNV studies,.

CAKUT occur as sporadic diseases in about 90% of subjects suggesting that de novo dominant mutations or recessive inheritance may account for a significant proportion of these cases. Until now, the majority of pathogenic genes in CAKUT follow an autosomal dominant inheritance pattern (**Chapter 1**). With whole-exome sequencing now performed in research as well as clinical settings, it will be more feasible to also identify recessive genes in individuals with a solitary functioning kidney. The novel candidate gene *TRAP1* is presented as an example for this in this thesis (**Chapter 11**).

Besides improving our understanding of renal malformations, genetic studies should also lead to the development of an individualized risk profile for children with a solitary functioning kidney, for example by associating the genetic make-up with the clinical outcome of cases. One example for this is the development of a genetic risk score for IgA nephropathy, in which the presence (or absence) of specific single nucleotide polymorphisms ("risk SNPs") increases the risk for IgA nephropathy.[382] Although the genetic backgrounds of IgA nephropathy and CAKUT seem not directly interchangeable, it is important for geneticists and nephrologists to develop genotype-phenotype correlation tools. Recently, the emerging role of genetic analyses in the diagnosis of CAKUT has been reported in a girl with a novel large deletion on chromosome 3.[334]

Another important example in the development of an individualized risk profile for solitary functioning kidney patients using genetic data is the high overlap between CNVs found in renal hypodysplasia patients and neurodevelopmental disorders.[21] Many children with complex CAKUT show neurodevelopmental delay or neurocognitive defects such as autism or attention deficit hyperactivity disorder. A substantial proportion of these patients indeed harbors genomic disorders caused by detectable pathogenic CNVs. Nowadays, the majority of CAKUT are prenatally identified, whereas many neurocognitive and neurodevelopmental defects do not come to clinical expression within the first years of life. Theoretically, a (prenatal) CNV screen therefore would allow identification of such complications before they become clinically evident as well as appropriate genetic counseling of family members and ultimately, therapeutic intervention.

Furthermore, expression of specific urinary miRs in urine have been associated with progression of autosomal dominant polycystic kidney disease and acute kidney-transplant rejection.[383,384] It only seems a matter of time before such miR profiling studies will be performed in CAKUT patients. However, to infer the outcomes of such studies to clinically usable risk profiles, we must establish longitudinal follow-up studies in a homogeneous cohort of CAKUT patients.

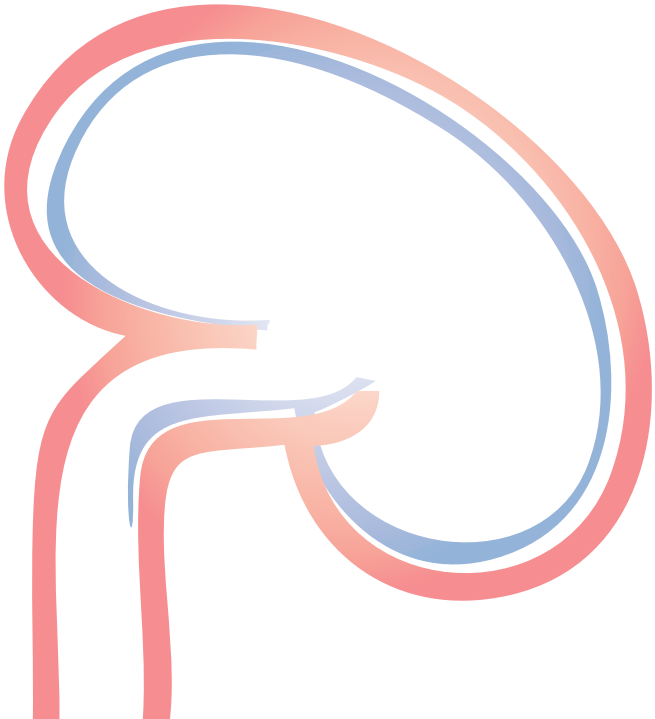
Finally, detection of epigenetic changes (through histone modification) in the genome may also help to understand the exact mechanisms of developmental disorders such as CAKUT. Chromatin profiling and epigenome analysis are powerful tools for annotating gene regulatory regions that may play a role in renal (mal)development.[385] Moreover, epigenetic findings may have implications for future therapy in kidney development. For example, inhibitors of the histone deacetylases (HADCi) are currently used in *ex vivo* studies to recapitulate nephrogenesis.[386] In E13.5 mice kidneys, long-term treatment (>12 hours) with HDACi resulted in arrested nephrogenesis and branching of the UB, whereas cell proliferation and cell survival was unaffected after shorter-term (<6 hours) treatment.[387] Therapeutic effects of HDACi have been observed in *PKD1* and *PKD2* mice, implicating that these agents are candidate drugs in the treatment of autosomal dominant polycystic kidney disease, and potentially, other cystic kidney diseases.[386]

FINAL CONCLUSIONS

A substantial proportion of children with a solitary functioning kidney have an increased risk for developing renal dysfunction. The trajectory for disease progression toward CKD starts in childhood and increases after puberty. Risk factors for the development of renal injury are ipsilateral urinary tract anomalies associated with the solitary functioning kidney, a small renal size and increasing age. As the diagnosis of a solitary functioning kidney nowadays is predominantly made by routine fetal ultrasound, an evaluation of risk factors and the clinical monitoring of individuals with a solitary functioning kidney should therefore start *in utero*. In children with complex CAKUT and/or extra-renal anomalies, state-of-the-art genetic studies allow clinicians to establish a molecular diagnosis, to inform family members and ideally, to reduce the risk for CKD by therapeutic interventions. Regular clinical monitoring requires a multidisciplinary approach in which evaluation for hypertension, moderately increased albuminuria and a decline in GFR is of cardinal importance. Ineffective transition from a pediatric to an adult nephrology setting of young adults should be prevented, as this may increase the risk for CKD. Furthermore, effective longitudinal follow-up programs will establish large cohort studies on the long-term outcome of adults with a solitary functioning kidney. High-throughput genetic studies and the development of *in vivo* techniques to determine

nephron number are essential to establish an individualized risk profile for children with solitary functioning kidneys. An important future task lies in the identification of new therapeutic targets and the development of new therapeutic strategies to reduce the risk for long-term effects of renal mass reduction, and, ultimately, prevent disease onset and progression in all children with this intriguing renal malformation.

Summary & Samenvatting



SUMMARY

Congenital anomalies of the kidney and urinary tract are the leading cause of end-stage renal disease in childhood. The solitary functioning kidney represents an important condition among the broad spectrum of urinary tract malformations, and has been associated with an impaired renal prognosis. Nevertheless, evidence-based clinical guidelines for individuals with solitary functioning kidneys have not been established.

To study the clinical outcome of solitary functioning kidney, the following research questions are addressed in this thesis:

1. What is the incidence of a solitary functioning kidney in childhood?
2. What is the incidence of renal injury in children with a solitary functioning kidney?
3. What are the risk factors for renal injury in children with a solitary functioning kidney?
4. Are estimating equations for GFR and office blood pressure measurement accurate applications in the clinical monitoring of children with a solitary functioning kidney?
5. What known and novel genetic factors can be identified in children with a solitary functioning kidney?

The rationale behind this thesis is further described in **Chapter 1**. This chapter provides a comprehensive overview of human and animal studies on renal mass reduction from childhood. Furthermore, the introduction describes the well-defined glomerular hyperfiltration hypothesis, which may underlie the impaired renal outcome of children with a solitary functioning kidney. Finally, recommendations for the clinical management of these patients are presented. In **Chapter 2**, the aims and outline of this thesis are presented with a brief overview of the methodology used.

In **Chapter 3**, we performed a systematic review on associated malformations of the kidney and urinary tract as well as extra-renal anomalies in patients with unilateral renal agenesis. Unilateral renal agenesis is a frequent cause of the congenital type of a solitary functioning kidney. Based on 43 eligible studies, 32% of individuals had associated urinary tract malformations. Of these, vesicoureteral reflux was the most commonly found anomaly (24%). Extra-renal manifestations were identified in 31% of patients. Moreover, the general incidence of unilateral renal agenesis was determined by analysis of the reported literature, and calculated to be 1 in ~2,000 births.

In **Chapter 4**, a similar approach was used to determine additional urinary tract malformations and extra-renal anomalies in patients with a unilateral multicystic dysplastic kidney. Together with unilateral renal agenesis, the multicystic dysplastic kidney represents the spectrum of conditions leading to a congenital solitary functioning kidney. Based on data from 19 populations, the general incidence of a unilateral multicystic dysplastic kidney was determined to be 1 in ~4,300 births. Associated urinary tract mal-

formations were found in 31% of patients, with vesicoureteral reflux identified as the most frequent anomaly (20% of cases). Extra-renal anomalies were detected in 15% of individuals.

The incidence of renal dysfunction in children with a solitary functioning kidney was studied in **Chapter 5**. In this retrospective study, we identified signs of renal injury, defined as the presence of hypertension and/or albuminuria and the use of antihypertensive/antiproteinuric agents, in 32% of children with a solitary functioning kidney. Moreover, we demonstrated that glomerular filtration rate declines from the beginning of puberty onwards. This decline is even more pronounced when children had additional urinary tract malformations.

The prognosis of individuals with solitary functioning kidneys is often derived from the excellent outcome of adult uninephric kidney donors. However, fundamental differences exist between both patient groups, which make it inadequate to assume that a solitary functioning kidney from childhood is harmless. In **Chapter 6**, we describe these discrepancies and emphasize why it is highly desirable to be born with two kidneys.

In **Chapter 7**, renal injury development during childhood was investigated in the largest cohort of solitary functioning kidney patients known to date (n=407). By performing Kaplan-Meier analysis, we identified that 50% of the children had developed at least one sign of renal injury at a median age of 15 years. This prognosis is even further impaired when ipsilateral urinary tract anomalies are present (median age toward renal injury: 13 years). Moreover, risk factors for renal injury development were age, associated malformations of the kidney and urinary tract and a small renal size. Low birth weight and a history of urinary tract infections showed a trend to increase the risk for renal injury. These findings imply that children with a solitary functioning kidney require regular clinical follow-up. At the time of diagnosis, an evaluation of risk factors should be made in order to improve the clinical outcome.

Gender differences in the incidence of congenital anomalies of the kidney and the urinary tract have been well established. However, it is unclear whether these gender differences also impact the renal prognosis. Although there is a male predominance in the incidence of solitary functioning kidney patients, no differences in the outcome between boys and girls were identified (**Chapter 8**).

The measurement of true glomerular filtration rate is cumbersome and costly, and therefore not routinely performed in children. As an alternative, pediatricians use equations to estimate glomerular filtration rate in daily clinical care. However, these equations have exclusively been designed in children with two kidneys, and, thus, using them in solitary functioning kidney patients may be inaccurate. In **Chapter 9**, we tested six commonly used equations for glomerular filtration rate by comparing them to a true measurement of renal function. Five of these common equations can be safely used in the monitoring of renal function of children with a solitary functioning kidney.

Blood pressure is an important clinical parameter in the development of renal dysfunction of solitary functioning kidney patients. We presented the blood pressure profiles of 24-hour ambulatory measurements of children with a solitary functioning kidney and compared them to the commonly used office blood pressure measurement (**Chapter 10**). Our findings indicate that regular 24h-ambulatory measurement is recommended in the clinical follow-up of solitary functioning kidney patients.

Genetic factors are increasingly recognized as the cause of a solitary functioning kidney from childhood. We described the difficulties in the discovery of genes that are implicated in renal and urinary tract malformations in **Chapter 11**. Furthermore, we presented important recommendations in order to unravel the genetic architecture of congenital anomalies of the kidney and urinary tract.

In **Chapter 12**, we performed genetic studies on the incidence of structural variants in children with both types of a solitary functioning kidney. We demonstrate that a genomic disorder can be identified in 14% of study subjects. Although this proportion appears low, this finding is a relatively important addition to our current understanding of genetic disease in solitary functioning kidney patients. Furthermore, we prioritized new disease-causing genes for renal malformations by performing a systematic computational approach and determining expression levels of candidate genes in the embryonic mouse kidney. The findings from this study are an important first step in unraveling the molecular mechanisms of renal maldevelopment.

Conclusions from our studies as well as methodological considerations, recommendations for future research and clinical management are described in **Chapter 13**:

1. The incidence of a congenital solitary functioning kidney should be divided into unilateral renal agenesis: 1 in ~2,000 births; and unilateral multicystic dysplastic kidney: 1 in ~4,300 births.
2. Based on two systematic reviews of the literature, as well as the largest cohort study performed to date, 1 in 3 children with a solitary functioning kidney has at least one sign of renal injury in childhood.
3. Renal injury is associated with increasing age, the presence of additional congenital anomalies of the kidney and urinary tract and a small renal size. Low birth weight and urinary tract infections show a trend with renal injury.
4. Glomerular filtration rate should be monitored by equations that combine serum creatinine and serum cystatin C. If serum cystatin C is not available, the commonly used estimating equation by Schwartz et al. provides an appropriate alternative. The urinary clearance of creatinine however, should be abandoned. Office blood pressure measurement may miss hypertension in a substantial proportion of children. Therefore, we recommend regular determination of blood pressure profiles by 24h-ambulatory blood pressure monitoring.

5. Disease-causing mutations in common genes were absent in our cohort. However, copy-number disorders (deletions and duplications) play an important role in the development of renal malformations. By using a systematic approach, these deletions and duplications allow us to define new genetic causes for a solitary functioning kidney.

SAMENVATTING

Aangeboren afwijkingen van de nieren en urinewegen zijn de voornaamste oorzaak van eindstadium nierfalen op de kinderleeftijd. De functionele mononier is een belangrijke aandoening binnen het uitgebreide palet van aangeboren afwijkingen van de nieren en de urinewegen. Een mononier kan bij de geboorte ontstaan, maar kan ook worden verworven omdat één nier op de kinderleeftijd afunctioneel is geraakt. Bij beide typen functionele mononier lijkt er inderdaad een verhoogd risico te bestaan op chronisch nierfalen op latere leeftijd. Desalniettemin zijn er geen klinische richtlijnen beschikbaar voor deze patiëntengroep en ontbreekt een gestandaardiseerde wijze om deze kinderen te volgen.

In dit proefschrift onderzoeken wij de prognose van kinderen met een functionele mononier. Wij doen dit door de volgende onderzoeksvragen te stellen:

1. Wat is het voorkomen van de functionele mononier op de kinderleeftijd?
2. In hoeveel kinderen met een functionele mononier kunnen tekenen van nierschade worden aangetoond?
3. Wat zijn de risicofactoren voor het ontwikkelen van nierschade bij kinderen met een functionele mononier?
4. Zijn de standaard gebruikte klinische meetmethoden, zoals het schatten van de nierfunctie en het meten van de bloeddruk, toepasbaar bij kinderen met een functionele mononier?
5. Welke genetische factoren spelen een rol in het ontstaan van de functionele mononier?

De achtergronden van dit proefschrift zijn beschreven in **Hoofdstuk 1**. Hier wordt een overzicht gegeven van de tot nu toe uitgevoerde onderzoeken naar afname van functioneel nierweefsel bij dieren en mensen. Bovendien beschrijft dit hoofdstuk het mechanisme van glomerulaire hyperfiltratie. De langetermijneffecten van glomerulaire hyperfiltratie vormen de belangrijkste gedachte waarom kinderen met een functionele mononier mogelijk nierschade ontwikkelen. Ook stellen wij een klinische richtlijn voor om de gezondheidstoestand van kinderen met een functionele mononier te evalueren.

Hoofdstuk 2 bespreekt de opgestelde onderzoeksvragen en beschrijft beknopt welke methoden zijn gebruikt om deze vragen te beantwoorden.

In **Hoofdstuk 3** hebben wij, door de bestaande wetenschappelijke literatuur op een systematische manier te ordenen, het voorkomen van bijkomende aandoeningen van de nieren en de urinewegen van kinderen met eenzijdige afwezigheid van nierweefsel (nieragenesie) in kaart gebracht. Nieragenesie is een belangrijke oorzaak voor de functionele mononier van het aangeboren type. Uit onze analyse blijkt dat 1 op de 3 kinderen bijkomende afwijkingen in de nieren en urinewegen heeft naast de nieragenesie.

Bovendien heeft 31% van de kinderen afwijkingen in andere organen zoals het hart en de darmen. Tevens hebben wij het wereldwijde voorkomen van nieragenesie berekend. Eenzijdige nieragenesie komt ongeveer bij 1 op de ~2.000 geboorten voor.

Op eenzelfde wijze als in **Hoofdstuk 3** hebben wij het bestaan van bijkomende aandoeningen van de nieren en urinewegen en andere orgaansystemen bestudeerd bij patiënten met eenzijdige multicysteuze nierdysplasie (**Hoofdstuk 4**). Naast eenzijdige nieragenesie is de multicysteuze nierdysplasie de andere uitingsvorm van een aangeboren functionele mononier. Uit onze analyse blijkt dat deze aandoening ongeveer 1 op de 4.300 geboorten voorkomt. Bovendien heeft 31% van de aangedane kinderen bijkomende aangeboren afwijkingen van de nieren en urinewegen, terwijl 15% afwijkingen heeft in andere organen.

In **Hoofdstuk 5** hebben wij onderzocht hoeveel kinderen met een functionele mononier tekenen hadden van nierschade. Onze definitie van nierschade betrof de aanwezigheid van hoge bloeddruk en/of de aanwezigheid van eiwitten in de urine op het moment van het onderzoek. Wanneer kinderen medicijnen gebruikten tegen hoge bloeddruk of tegen eiwit in de urine, dan werd dit ook beschouwd als nierschade. Door klinische gegevens over deze kinderen te verzamelen, kon worden beschreven dat 32% van hen tenminste één uitingsvorm van nierschade heeft. Bovendien blijkt de nierfunctie vanaf het begin van de puberteit bij deze kinderen langzaam af te nemen. Met name wanneer bijkomende afwijkingen van de nieren en urinewegen aanwezig waren, blijkt deze verslechtering van de nierfunctie extra uitgesproken.

Hoofdstuk 6 bespreekt de verschillen tussen individuen met een functionele mononier en volwassenen die een nier hebben afgestaan ter donatie. De goede prognose van deze nierdonoren wordt vaak vergeleken met kinderen met een functionele mononier. Deze vergelijking is niet correct, omdat een functionele mononier, in tegenstelling tot de overgebleven nier van gezonde volwassen nierdonoren, voortkomt uit een aanlegstoornis van de urinewegen. Dit betekent dat de functionele mononier zelf ook verstoord kan zijn in haar aanleg.

Eventueel aanwezige risicofactoren bij patiënten helpen hun dokters om in een vroeg stadium een inschatting te maken welke kinderen met een functionele mononier een hoger risico hebben op nierschade, en bij welke kinderen een lager risico bestaat. Wij hebben deze risicofactoren opgesteld in de grootste groep van kinderen met een functionele mononier ter wereld. In **Hoofdstuk 7** hebben wij aangetoond dat toenemende leeftijd, de aanwezigheid van bijkomende afwijkingen aan de nieren en urinewegen en een geringere nierlengte een groter risico op nierschade inhouden. Ook een laag geboortegewicht en een ziektegeschiedenis met urineweginfecties lijken de prognose te verslechteren. Bovendien blijkt 50% van de kinderen op een mediane leeftijd van 15 jaar tenminste een van de tekenen voor nierschade te hebben. Dit is een zorgelijk gegeven wanneer in ogenschouw wordt genomen dat de nier van deze patiënten nog het gehele

leven adequaat moet functioneren. Het lijkt dus zeer belangrijk om de gezondheidstoestand van deze kinderen gedurende het leven te evalueren.

Het is bekend dat jongens vaker afwijkingen van de nieren en urinewegen hebben dan meisjes. Het is echter niet bekend of er geslachtsverschillen bestaan in de prognose van de functionele mononier. Dit wordt besproken in **Hoofdstuk 8**, waar wij geen verschillen hebben kunnen aantonen in de aanwezigheid van nierschade tussen jongens en meisjes.

Het meten van de ware nierfunctie is kostbaar, arbeidsintensief en vereist een infuus bij het kind. Deze test is daarom niet standaard beschikbaar in de dagelijkse praktijk. Om dit te ondervangen worden doorgaans schattingsformules voor de nierfunctie gebruikt. Deze formules zijn echter ontwikkeld voor kinderen met twee nieren, en het is daarmee onduidelijk of zij toepasbaar zijn bij kinderen met een functionele mononier. In **Hoofdstuk 9** hebben wij zes veelgebruikte schattingsformules voor de nierfunctie getest in deze patiëntengroep door de schattingen te vergelijken met de ware nierfunctiemeting. Vijf van de zes formules bleken nauwkeurig genoeg om de nierfunctie van kinderen met een functionele mononier te schatten.

De bloeddruk is een belangrijke maatstaf voor het ontwikkelen van nierfalen bij kinderen met een functionele mononier. Door 24-uurs bloeddrukmetingen te verrichten, zijn de bloeddrukprofielen over de dag en nacht van deze kinderen bepaald (**Hoofdstuk 10**). Bovendien vergeleken wij deze bloeddrukprofielen met de normaal gebruikte bloeddrukmeting op de polikliniek. Om een verhoogde bloeddruk tijdig te signaleren, lijkt het belangrijk te zijn om regelmatig 24-uurs bloeddrukmetingen te verrichten bij deze kinderen.

Het ontstaan van de functionele mononier wordt toenemend toegeschreven aan afwijkingen in het erfelijk materiaal (DNA). Het is echter zeer moeilijk gebleken om het complexe netwerk van genetische afwijkingen van aangeboren afwijkingen van de nieren en urinewegen te ontrafelen. **Hoofdstuk 11** beschrijft de beperkingen in het verrichten van genetische studies voor deze aandoening en presenteert aanbevelingen voor toekomstig onderzoek.

In **Hoofdstuk 12** bestuderen wij de structurele afwijkingen in het erfelijk materiaal bij kinderen met beide typen functionele mononier. Door de aanwezigheid van deleties en duplicaties (respectievelijk afwezigheid en verdubbeling van delen van het erfelijk materiaal) in het DNA van deze kinderen te onderzoeken, is het mogelijk om een genetische oorzaak te vinden in 14% van de patiënten met een functionele mononier. Hoewel dit op het eerste oog de minderheid van de onderzochte kinderen betreft, is deze bevinding van een zeer grote toevoegende waarde voor onze huidige opvattingen over genetische nierafwijkingen. Zo maakt onze analyse het mogelijk om zogenaamde kandidaatgenen aan te wijzen die mogelijk een rol spelen in het ontstaan van de functionele mononier.

Daarmee vormen onze bevindingen een belangrijke stap in het in kaart brengen van de ontstaanswijze van de functionele mononier.

In **Hoofdstuk 13** worden onze studieresultaten in een breder perspectief geplaatst en worden er tevens voorstellen gedaan voor toekomstige onderzoeken bij kinderen met een functionele mononier. Bovendien bevat dit hoofdstuk de belangrijkste conclusies van dit proefschrift:

1. Het voorkomen van de aangeboren functionele mononier wordt onderverdeeld in eenzijdige nieragenesie: 1 op ~2.000 geboorten; en eenzijdige multicysteuze nierdysplasie: 1 op ~4.300 geboorten.
2. Op 9-jarige leeftijd heeft 1 op de 3 kinderen met een functionele mononier tekenen van nierschade.
3. Risicofactoren voor het ontwikkelen van nierschade bij kinderen met een functionele mononier zijn leeftijd, de aanwezigheid van aangeboren afwijkingen van de nieren en urinewegen en een geringere nierlengte.
4. De nierfunctie bij kinderen met een functionele mononier dient geschat te worden door middel van een schattingsformule die gebruik maakt van laboratoriumwaarden van creatinine en cystatine C in het bloed. Als cystatine C niet kan worden bepaald, dan kan de herziene standaard schattingsformule van Schwartz et al. worden gebruikt. De creatinineklaring in 24-uurs urine is echter niet precies genoeg om de nierfunctie te voorspellen. Het is bovendien belangrijk om de bloeddruk van deze patiëntengroep te bepalen met regelmatige 24-uurs bloeddrukmetingen.
5. Structurele afwijkingen (deleties en duplicaties) in het erfelijk materiaal zijn een relatief veel voorkomende oorzaak voor de functionele mononier. Door middel van een systematische analyse kunnen bovendien nieuwe genen worden gevonden die van belang zijn in de ontwikkeling van een functionele mononier.

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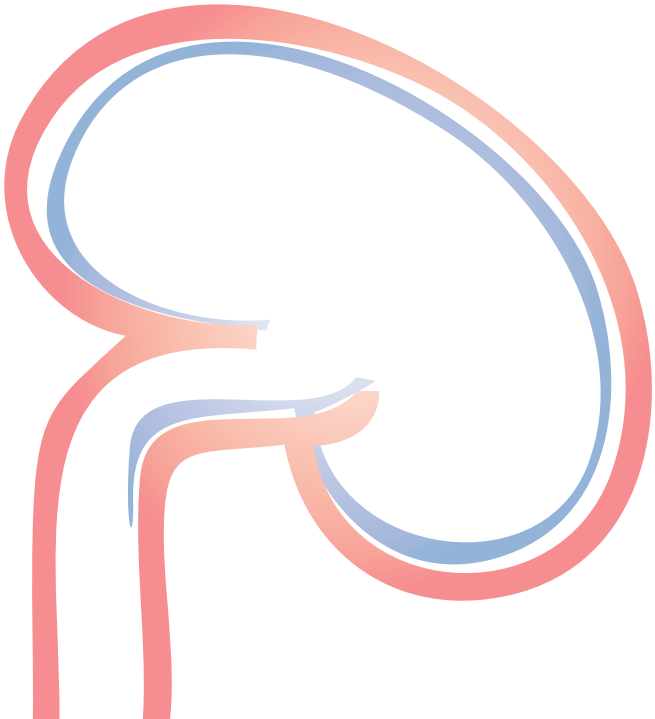

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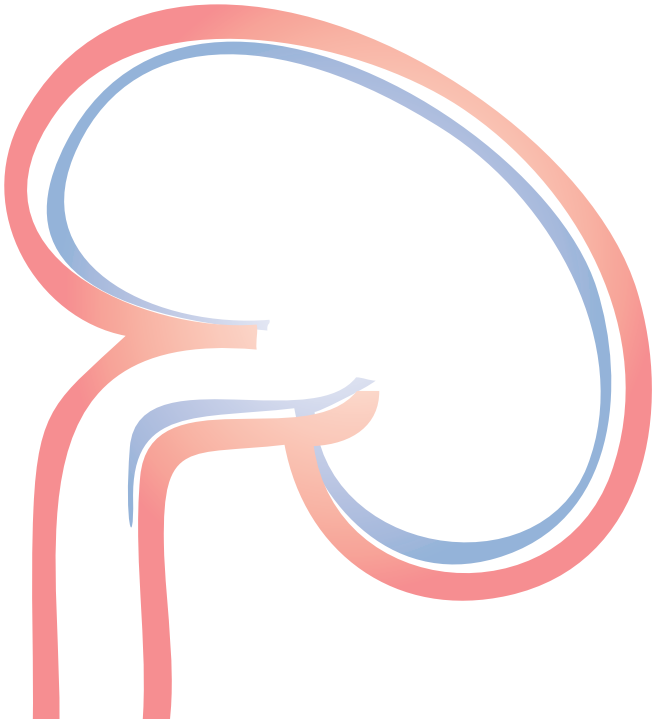
List of Publications



THIS THESIS

Westland R, Verbitsky M, Vukojevic K, Perry BJ, Fasel DA, Zwijnenburg PJG, Bökenkamp A, Gille JJP, Mendelsohn CL, Ghiggeri GM, Mendelsohn CL, Ghiggeri GM, D'Agati VD, Gharavi AG, Schreuder MF, van Wijk JAE, Sanna-Cherchi S. Copy-number analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney from the KIMONO-study. *Submitted*

Westland R, Schreuder MF, van der Lof DF, Vermeulen A, Dekker-van der Meer IMJ, Bökenkamp A, van Wijk JAE. Ambulatory blood pressure monitoring is recommended in the clinical management of children with a solitary functioning kidney. *Pediatr Nephrol* 2014 Jun 9. [Epub ahead of print]

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Westland R, Abraham Y, Bökenkamp A, Stoffel-Wagner B. Schreuder MF, van Wijk JAE. Precision of estimating equations for GFR in children with a solitary functioning kidney: The KIMONO Study. *Clin J Am Soc Nephrol* 2013 May;8(5):764-72

Westland R, Schreuder MF. Gender differences in solitary functioning kidney: do they affect renal outcome? *Pediatr Nephrol* 2013 Apr 11. [Epub ahead of print]

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OTHER PUBLICATIONS

Westland R, Bodria M (equal contribution), Carrea A, Lata S, Scolari F, D'Agati VD, Lifton RP, Gharavi AG, Ghiggeri GM, Sanna-Cherchi S. Phenotypic expansion of DGKE-associated diseases. *J Am Soc Nephrol* 2014 Feb 7. [Epub ahead of print]

Blufpand HN, Westland R (equal contribution), van Wijk JAE, Roelandse EA, Kaspers GJ, Bökenkamp A. Height-independent estimation of GFR in children: an alternative for the Schwartz equation. *J Pediatr* 2013 Dec;163(6):1722-7

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Westland R, Schreuder MF, van Wijk JAE. Nierschade bij kinderen met een mononier. *Tijdschrift voor Kindergeneeskunde* 2012 Dec;6:129-136

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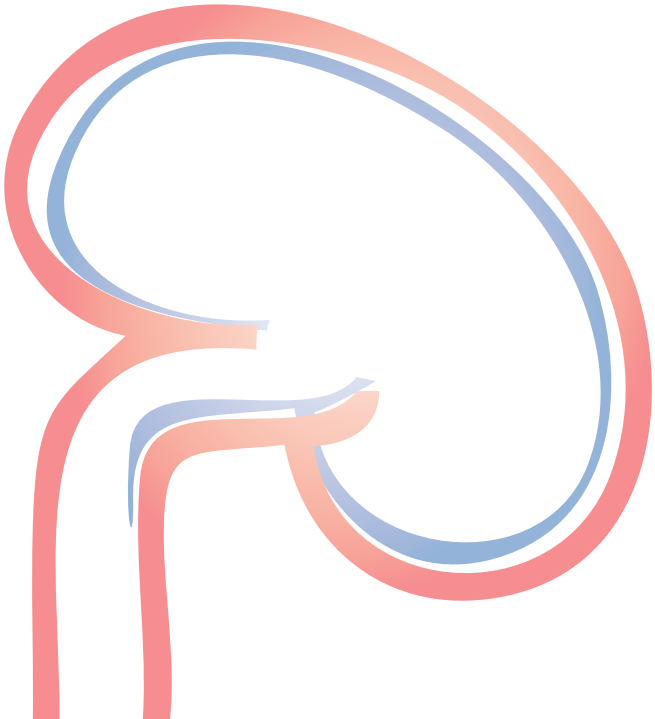
Curriculum Vitae



Rik Westland was born on November 8, 1985 in Eemnes, the Netherlands. In 2004, he graduated from secondary school at Christelijk College Nassau-Veluwe in Harderwijk. In the same year, he started medical school at the VU University Medical Center in Amsterdam from which he graduated *cum laude* in 2010.

Pediatrics and Science were his *core business* ever since he started medical school. In his second year, he got accepted into the Honours Programme Medicine of the VU University Medical Center in Amsterdam (supervisors: dr. J.A.E. van Wijk & dr. I.J. van Wijk), a three-year research program for medical students. This program allowed him to pursue his two interests by combining them into a translational research project. In addition to conducting research, he participated in the board of the Honours Programme Medicine as a student-member. For his Honours Programme project, he won the Young Investigator Award of the Department of Pediatrics of the VU University Medical Center in Amsterdam in 2010.

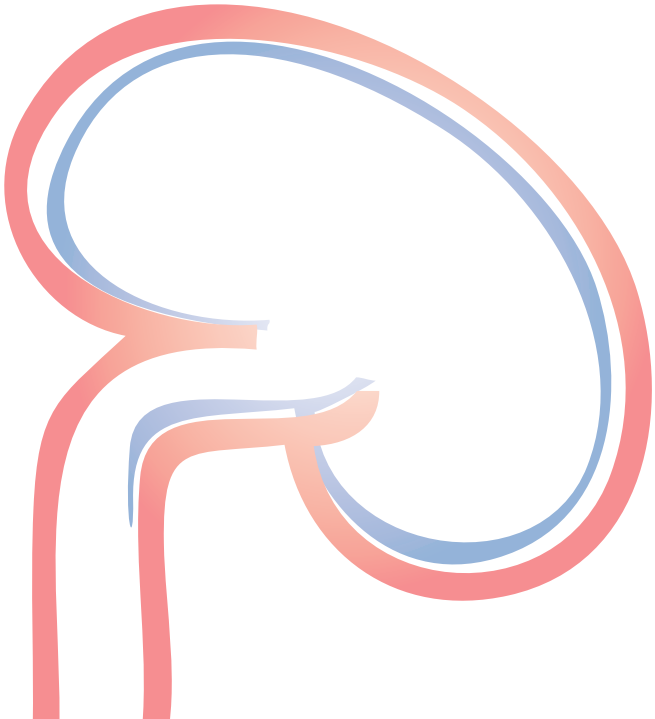
Between December 2010 and January 2012, he worked as a resident (ANIOS) at the Department of Pediatrics of the Westfriesgasthuis in Hoorn (mentor: dr. G.W. Ten Tusscher), and the Department of Pediatrics of the VU University Medical Center in Amsterdam (mentor: drs. E. Edelenbos). During this year, he was able to realize his dream by expanding his Honours Programme Medicine studies into the PhD project described in this thesis.

He officially started this PhD program in January 2012 (mentors: dr. J.A.E. van Wijk, dr. M.F. Schreuder and prof. dr. J.B. van Goudoever). His PhD project was awarded grants from Fonds NutsOhra Zorgsubsidies, the Royal Netherlands Academy for Arts and Sciences (KNAW) Ter Meulen Grants, and Pfizer Netherlands. From January until November 2013, he trained at the renowned laboratory headed by A.G. Gharavi, M.D., Columbia University College of Physicians and Surgeons, New York, USA, under the supervision of S. Sanna-Cherchi, M.D., in dissecting the genetic basis of congenital renal malformations.

Shortly after his return to the Netherlands, he was selected as a member of the Global Shapers Community of the World Economic Forum, an international network of young leaders who are driven to make a contribution to their communities, and got accepted into the Pediatric residency training program of the VU University Medical Center in Amsterdam (program director: prof. dr. R.J.B.J. Gemke). He started his training in Pediatrics at Onze Lieve Vrouwe Gasthuis in Amsterdam (program director: dr. A.A.M.W. van Kempen) in April 2014.

Rik is together with Tessa. They live in Amsterdam.

Dankwoord - Acknowledgments



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